On the synthesis of some chiral phosphoinositide ligands from carbohydrates

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On the Synthesis of Some Chiral Phosphoinositide Ligands from Carbohydrates

A Thesis submitted by David John Jenkins
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ABSTRACT

Syntheses of some chiral analogues of the second messenger D-*myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3], using carbohydrates as starting materials, are described.

Methyl α-D-glucopyranoside was converted by an improved procedure to methyl 4,6-*O*-benzylidene-α-D-glucopyranoside and thence to methyl 3-*O*-benzoyl-2-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside without recourse to column chromatography. The latter compound was converted to methyl 3,4-di-*O*-benzoyl-2-*O*-benzyl-6-deoxy-α-D-*xylo*-hex-5-enopyranoside *via* methyl 3,4-di-*O*-benzoyl-2-*O*-benzyl-6-bromo-6-deoxy-α-D-glucopyranoside. Rearrangement of the hex-5-enopyranoside with mercury (II) trifluoroacetate provided (2*S*, 3*R*, 4*S*, 5*R*)-2,3-dibenzoyloxy-4-benzyloxy-5-hydroxycyclohexanone and (2*S*, 3*R*, 4*S*, 5*S*)-2,3-dibenzoyloxy-4-benzyloxy-5-hydroxycyclohexanone. Attempts to invert the configuration at position 5 of the latter were unsuccessful, but provided a number of discrete products. Reduction of (2*S*, 3*R*, 4*S*, 5*R*)-2,3-dibenzoyloxy-4-benzyloxy-5-hydroxycyclohexanone and saponification furnished D-1-*O*-benzyl-3-deoxy-*scyllo*-inositol, which was phosphorylated and deprotected to give the first target compound, D-2-deoxy-*myo*-inositol-1,3,4,5-tetrakisphosphate.

Methyl α-D-glucopyranoside was protected as its 4,6-*O*-(*p*-methoxybenzylidene) derivative. Regioselective benzylation followed by *p*-methoxybenzylation and reductive cleavage of the acetal furnished methyl 2-*O*-benzyl-3,4-di-*O*-(*p*-methoxybenzyl)-α-D-glucopyranoside. This compound was oxidised to the corresponding aldehyde which was subjected to a samarium (II) iodide-catalysed ring contraction to yield (1*R*, 2*S*, 3*S*, 4*R*, 5*S*)-3-benzyloxy-4-hydroxy-5-hydroxymethyl-1,2-di-(*p*-methoxybenzyloxy)cyclopentane. This compound was benzylated and hydrolysed under acidic conditions to give (1*R*, 2*S*, 3*S*, 4*R*, 5*R*)-3-benzyloxy-5-benzyloxymethyl-1,2,4-trihydroxycyclopentane, which was further elaborated to furnish the second target compound, (1*R*, 2*R*, 3*S*, 4*R*, 5*S*)-3-hydroxy-5-hydroxymethyl-1,2,4-trisphosphocyclopentane.

Methyl α-D-glucopyranoside was converted to (*E*,*Z*)-methyl 6-*O*-acetyl-3,4-*O*-isopropylidene-2-*O*-(*p*-methoxybenzyl)-α-D-*xylo*-hex-5-enopyranoside, which failed to yield a cyclohexanone when treated with mercury (II) acetate in aqueous acetone.

An anomeric mixture of allyl glucopyranosides was obtained by Fischer glycosylation and the α-anomer was isolated by crystallisation. Treatment of the anomeric mixture with 1.05 equiv. dibutyltin oxide followed by 2.1 equiv. of benzoyl chloride gave allyl 2,6-di-*O*-benzoyl-α-D-glucopyranoside, which was converted in 3 steps to allyl 2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-α-D-glucopyranoside. Alternatively, treatment of the pure α-anomer with 2.5 equiv. of dibutyltin oxide followed by benzyl bromide gave allyl 2,6-di-*O*-benzyl-α-D-glucopyranoside which was also converted to the 3,4-*O*-isopropylidene derivative. Allyl 2,6-di-*O*-benzyl-α-D-glucopyranoside was converted to 2,6-di-*O*-benzyl-3,4-di-*O*-(*p*-methoxybenzyl)-D-glucopyranose, a useful intermediate for the synthesis of adenophostin A and related compounds.

Allyl 2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-α-D-glucopyranoside was elaborated to the phosphorylation precursor (2-hydroxyethyl) 2,6-di-*O*-benzyl-α-D-glucopyranoside in a convenient one-pot reaction, and this precursor was phosphorylated and deblocked to afford the third target polyphosphate (2-hydroxyethyl) α-D-glucopyranoside 2',3,4-trisphosphate.

Allyl 2,6-di-*O*-benzyl-α-D-glucopyranoside was converted in four steps to (2-hydroxyethyl) α-D-glucopyranoside 3,4-bisphosphate-2'-phosphorothioate, which was attached to a BODIPY FL fluorescent label.

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DEDICATION

To Sheelagh, with love.

"A friend may well be reckoned the masterpiece of nature"

EMERSON, "Friendship," *Essays: First Series* (1841)

ABBREVIATIONS

Ac	acetyl
ADP	adenosine 5'-diphosphate
All	allyl
AMP	adenosine 5'-monophosphate
Ar	aryl
ATP	adenosine 5'-triphosphate
Bn	benzyl
BODIPY FL	*N*-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-*s*-indacene-3-propionyl
BOM	benzyloxymethyl
Bz	benzoyl
cAMP	adenosine 3',5'-cyclic phosphate
cGMP	guanosine 3',5'-cyclic phosphate
°C	degrees Celcius
Cbz	carbobenzyloxy
COSY	correlated spectroscopy
DAG	1,2-diacylglycerol
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DEAD	diethyl azodicarboxylate
DEPT	distortionless enhancement by polarisation transfer
DMAP	*N*,*N*-4-dimethylaminopyridine
DMSO	dimethylsulphoxide
DMF	*N*,*N*-dimethylformamide
EC_{50}	concentration of entity producing 50% of maximum response
Et	ethyl
FAB	fast atom bombardment
GDP	guanosine 5'-diphosphate
GMP	guanosine 5'-monophosphate
GTP	guanosine 5'-triphosphate

h	hour
HMPA	hexamethylphosphoric triamide
IR	infra-red
K_i	dissociation constant for an inhibitor-enzyme complex
MCPBA	*m*-chloroperoxybenzoic acid
Me	methyl
min	minute(s)
MOM	methoxymethyl
NMR	nuclear magnetic resonance
PCB	*p*-chlorobenzyl
Ph	phenyl
PLC	phospholipase C
PMB	*p*-methoxybenzyl
Prop	prop-1-enyl
PTSA	*p*-toluenesulphonic acid
TEAB	triethylammonium bicarbonate
THF	tetrahydrofuran
THP	tetrahydropyran-2-yl
TLC	thin layer chromatography
Ts	tosyl
UV	ultra-violet

CHAPTER TWO: SYNTHESIS OF D-2-DEOXY-*myo*-INOSITOL-1,3,4,5-TETRAKISPHOSPHATE

CHAPTER ONE

INTRODUCTION

1.1 Transmembrane Signalling Systems

Intercellular communication is essential in multicellular organisms to co-ordinate the diverse activities of different cell types. In mammals such communication occurs by the endocrine and paracrine systems, and neurotransmission. Hormones and neurotransmitters, once released, often bind to receptors on the outer surface of plasma membranes of their target cells.

With the exception of steroid and thyroid hormones, which are internalised, these hydrophilic transmitters are unable to cross the hydrophobic plasma membranes. Therefore a transduction mechanism is required to couple the incoming signal to an intracellular response.

Three main classes of plasma membrane receptor have been identified:

i) Those which are intrinsically ion channels. On stimulation, a dramatic alteration in their permeability to ions occurs within fractions of a millisecond and a rapid entry of ions into the cells occurs. This system is by far the fastest transduction process and has evolved where the swiftest response to the extracellular stimulus is required, *e.g.* in nervous impulse conduction. An example is the nicotinic acetylcholine receptor[1] found on postsynaptic membranes of cholinergic synapses. In response to acetylcholine the postsynaptic neurone is depolarised and an action potential is initiated in the adjacent axon or muscle membrane.

ii) Those which are intrinsically tyrosine kinase enzymes. These proteins span the membrane and binding of the extracellular agonist alters the conformation of the kinase and causes pairs of receptors to dimerise. The association of the two intracellular kinase domains results in autophosphorylation of tyrosine residues in these domains. The autophosphorylated tyrosine residues then tightly bind to phosphotyrosine residues of particular intracellular receptors, causing activation of enzymes or transcription factors. This transduction system is relatively slow and tends to be the effector for growth factors and the longer-acting hormones, such as insulin.[2]

iii) Those whose transduction mechanism occurs *via* GTP-binding ("G-") proteins. This class, termed "metabotropic" receptors, is schematically represented in fig. 1.1. Binding of an agonist (the "first messenger") to its extracellular receptor (step 1) stimulates a G-protein to release a molecule of GDP and bind GTP (step 2). The G-protein then activates a membrane-bound enzyme to catalyse the release of the "second messenger" (step 3). There are many subtypes of G-protein[3] and several second messengers have been identified. These are adenosine 3',5'-cyclic monophosphate (cAMP, **1**) (reviewed[4]) and guanosine 3',5'-cyclic monophosphate (cGMP, **2**) (reviewed[5]) which activate protein kinases, D-*myo*-inositol-1,4,5-trisphosphate* [Ins(1,4,5)P_3, **3a**] which mobilises Ca^{2+} from intracellular stores,[6] and diacylglycerol (DAG, **4**) (reviewed[7]) which activates protein kinase C. In addition, two putative second messengers are phosphatidylinositol 3,4,5-trisphosphate [PtdIns(3,4,5)P_3, **5**] (reviewed[8]) and cyclic adenosine diphosphate ribose (cADP-ribose, **6**) (reviewed[9]).

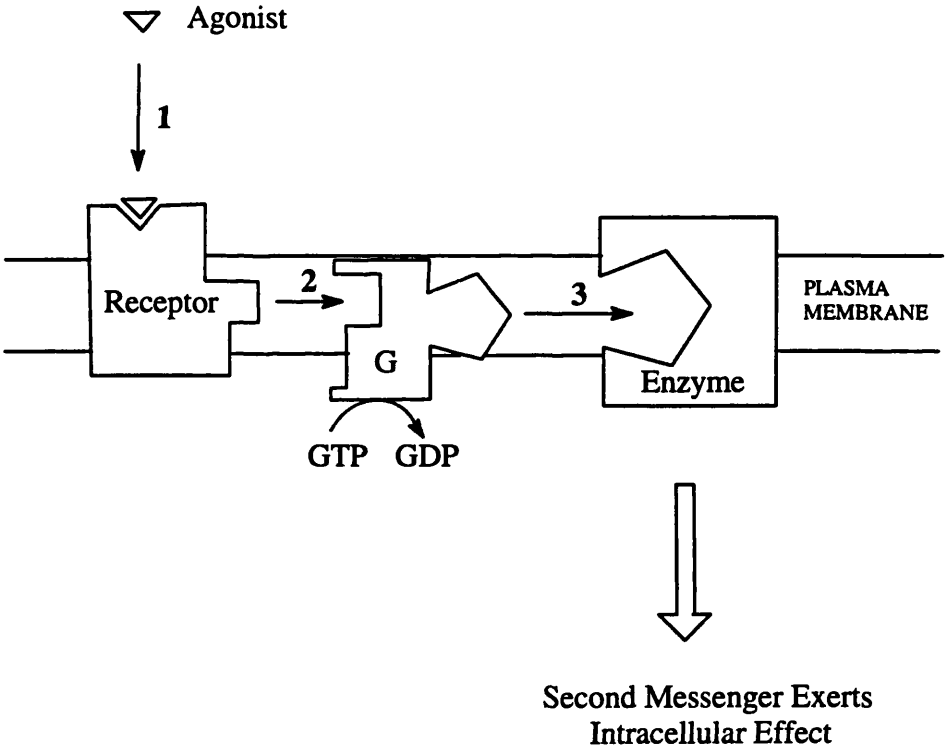
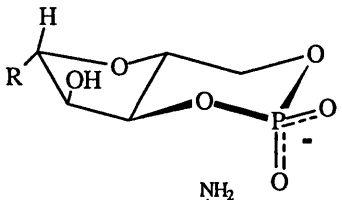
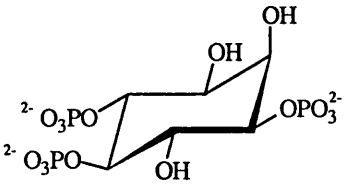


Fig. 1.1 Schematic representation of G-protein-mediated transmembrane signalling.

*See Appendix for nomenclature and numbering of inositols and derivatives.





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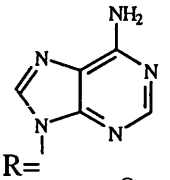

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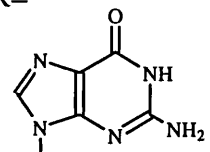
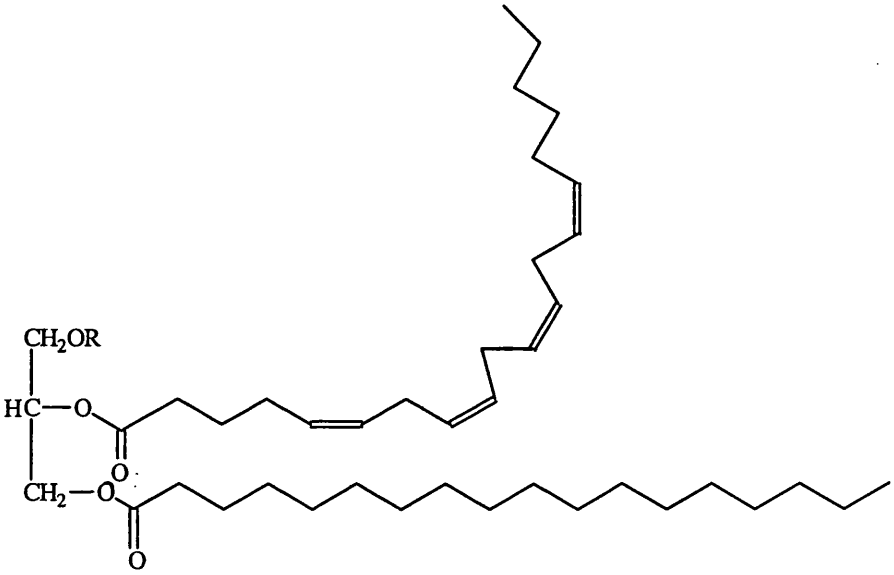
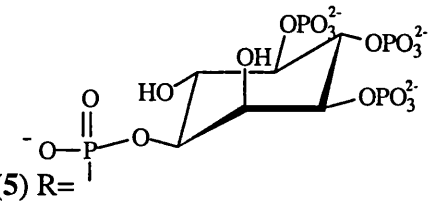


(2) R=



(4) R=H



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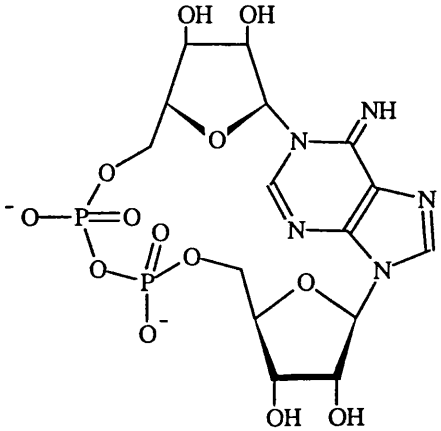

(6)

Two important consequences of transduction (in addition to variation of speed of response) are as follows: First, one molecule of extracellular agonist can cause many molecules of second messenger to be produced, *i.e.* there is an amplification of the signal across the cell membrane. This amplification leads to an extremely sensitive system as relatively small changes in the concentration of liberated second messenger have an enormous impact upon cell physiology. Second, the same extracellular agonist can elicit a markedly different response in different tissues. This may occur either a) by utilisation of different classes of plasma membrane receptor, *e.g.* acetylcholine nicotinic receptors are of the ion channel type while muscarinic receptors are coupled to G-protein systems or b) by utilising different second messengers, *e.g.* the H_1-histaminic receptor is coupled to Ins(1,4,5)P_3 production, whereas the H_2-receptor is coupled to cAMP production.[10] In addition, heterogeneity in G-proteins and potential functional differences in second messenger receptor subtypes offer many possibilities for variation in type and degree of response. It should be emphasised that none of these systems operates in isolation. In healthy cells a delicate equilibrium exists between the various transduction systems.

The overall project in these laboratories, of which this thesis forms a part, is concerned with the study of the interaction of the second messenger Ins(1,4,5)P_3 with its intracellular receptor. An extensive review of the chemistry and biology of the Ins(1,4,5)P_3 signalling pathway has recently been published.[11] The remainder of this chapter will briefly introduce the phosphoinositide system. Two topics will be considered in detail: the Ins(1,4,5)P_3 receptor, because many biochemical advances have recently been reported; and structure-activity relationships at the receptor, which form the most important background for the work described in this thesis.

1.2 The Phosphoinositide System

1.2.1 Phospholipids

Inositol-containing phospholipids account for less than 10% of the total phospholipid in mammalian cells. The structures of phosphatidylinositol [PtdIns, **7**],

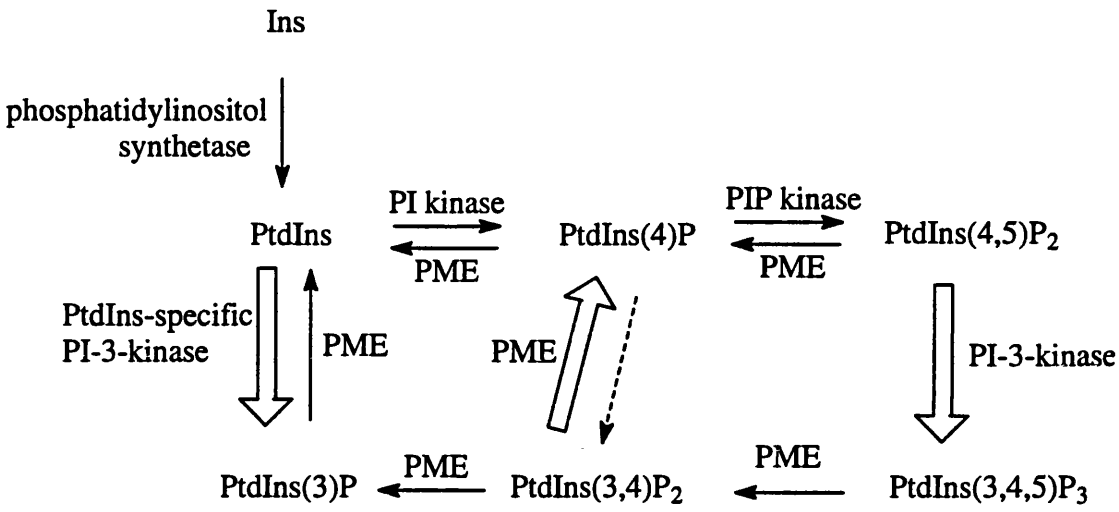

Fig. 1.2 Metabolism of phosphatidylinositol phosphates. PME=specific phosphomonoesterase.

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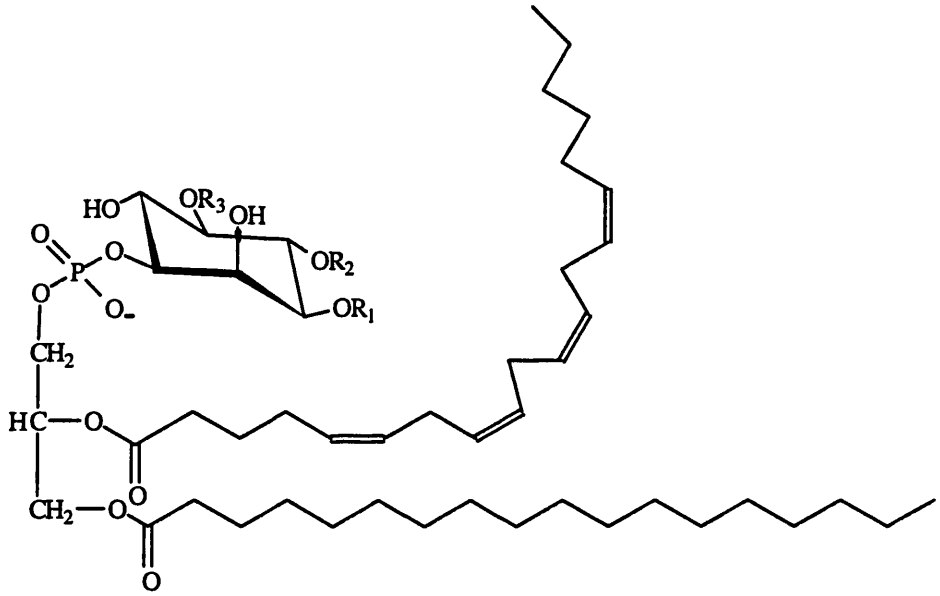


(5) $R_1=R_2=R_3=PO_3^{2-}$
(7) $R_1=R_2=R_3=H$
(8) $R_1=R_3=H$; $R_2=PO_3^{2-}$
(9) $R_1=H$; $R_2=R_3=PO_3^{2-}$
(10) $R_1=PO_3^{2-}$; $R_2=R_3=H$
(11) $R_1=R_2=PO_3^{2-}$; $R_3=H$

phosphatidylinositol 4-phosphate [PtdIns(4)P, **8**], and phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2, **9**] were elucidated by Brockerhoff and Ballou[12] in 1961, while more recently phosphatidylinositol 3-phosphate [PtdIns(3)P, **10**],[13] phosphatidylinositol 3,4-bisphosphate [PtdIns(3,4)P_2, **11**][14] and phosphatidylinositol 3,4,5-trisphosphate (**5**)[15] have been identified. The metabolic pathway controlling the interconversion of these compounds is summarised in fig. 1.2. In animals, the *sn*-1 glycerol carbon atom is usually linked to stearic acid and the *sn*-2 carbon atom to arachidonic acid, as shown. In plants, the *sn*-2 carbon is linked to linoleic acid and the *sn*-1 carbon to one of several fatty acids.[11]

PtdIns, which forms about 90% of the inositol-containing phospholipid in animals, is chiefly located in the endoplasmic reticulum. The other phospholipids described above occur on the inner leaflet of the plasma membrane.

1.2.2 Discovery of the Second Messenger Rôle of Ins(1,4,5)P_3

In 1953 Hokin and Hokin[16] demonstrated that acetylcholine stimulated the incorporation of [^{32}P]HPO_4^{2-} into phospholipids from pancreatic acinar cells. This was interpreted as acetylcholine stimulating the synthesis of phospholipid and subsequent studies indicated the major phospholipid to be PtdIns. Over twenty years later, Michell[17] noted that agonists which stimulated such phospholipid turnover also raised intracellular Ca^{2+} concentration and suggested these phenomena were linked. In 1983 Streb *et al.*[18] showed that Ins(1,4,5)P_3 was the phospholipid-derived second messenger responsible for mobilising intracellular Ca^{2+}.

It is now well established that on extracellular stimulation (step 1; fig. 1.3) the receptor activates a G-protein(step 2), which then stimulates phospholipase C to hydrolyse the minor membrane phospholipid PtdIns(4,5)P_2 into Ins(1,4,5)P_3 and DAG (step 3). Ins(1,4,5)P_3 diffuses into the cytosol, binds to its receptor and causes release of Ca^{2+} from the endoplasmic reticulum into the cytosol (step 4). Ins(1,4,5)P_3 is then inactivated by metabolism (step 5). Each of these five processes has been intensively studied, and will now be considered in a little more detail.

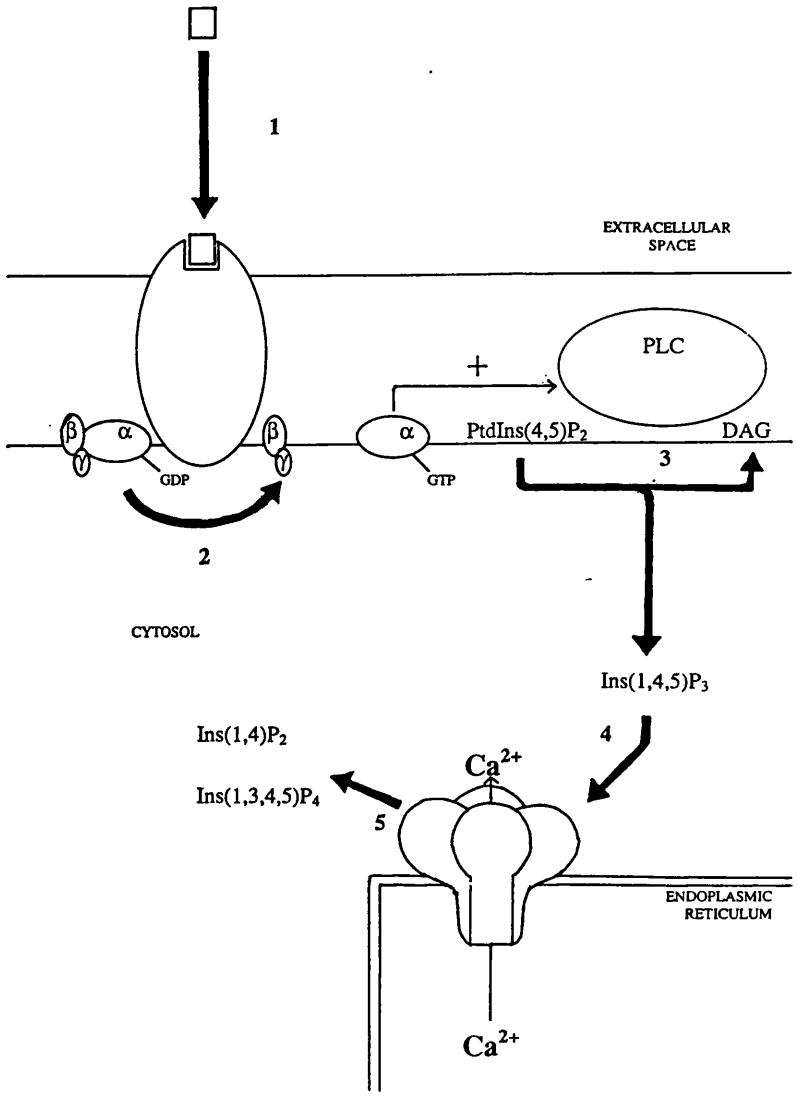


Fig. 1.3 Sequence of events in signal transduction pathways involving phosphoinositide turnover. Most of the effects of Ca^{2+} result from its binding to calmodulin.

1.2.3 Extracellular Receptors Coupled to $Ins(1,4,5)P_3$ Production

The fundamental importance of the phosphoinositide system is illustrated by table 1.1, which shows the range of different receptors known to be coupled to the pathway. All G-protein-linked receptors characterised so far exhibit the same general structure. They consist of a single polypeptide chain possessing seven regions rich in hydrophobic

amino acids which form the membrane-spanning domains. These transmembrane domains interact to provide the extracellular agonist binding site. In the case of phosphoinositide-coupled receptors, binding of the agonist induces a conformational change in the protein which causes the second and third cytoplasmic loops of the receptor to interact with and thereby activate an associated G-protein. The topic of receptor-G-protein interaction has been reviewed.[19]

Receptor Type	Endogenous ligand(s)
α_1-adrenoceptors	NA, adrenaline
Angiotensin AT_1	Angiotensin II
Bombesin BB_1 & BB_2	GRP, NMB
Bradykinin B_1 & B_2	Bradykinin, kallidin, T-kinin
Cholecystokinin & gastrin	Cholecystokinin, gastrin
Endothelin ET_A & ET_B	Endothelins 1–3
Glutamate $mGluR_1$ & $mGluR_5$	Glutamate
Histaminic H_1	Histamine
$5\text{-}HT_{2A-2C}$	5-HT
Leukotriene BLT & $CysLT_1$	LTB_4, LTC_4, LTD_4, LTE_4, (12*R*)-HETE (BLT), Lipoxin A_4 ($CysLT_1$)
Muscarinic M_1, M_3 & M_5	Acetylcholine
Neurotensin	Neurotensin, Neuromedin B
Prostanoid EP_1 & EP_3 FP TP	PG E_2 PG $F_{2\alpha}$ TxA_2
PAF	PAF
Purinoceptors P_{2Y} & P_{2U}	ATP, ADP, AMP, UTP (P_{2U})
Tachykinin NK_{1-3}	Substance P, Neurokinins A–B, Neuropeptides K, γ
Vasopressin V_{1A}, V_{1B} & OT	Vasopressin

Table 1.1. Receptors known to be coupled to phosphoinositide turnover. Compiled from *Trends Pharmacol. Sci.* 1995 Receptor and Ion Channel Nomenclature Supplement.[10] Abbreviations: NA, noradrenaline; GRP, gastrin releasing peptide; HETE, hydroxyeicosatetraenoic acid; NMB, neuromedin B; 5-HT, 5-hydroxytryptamine; LT, leukotriene; PAF, platelet activating factor; Tx, thromboxane.

1.2.4 G-Proteins

Signal transducing G-proteins are of two general types. "Small G-proteins" are involved in regulation of cell growth, protein secretion and intracellular vesicle interaction. Heterotrimeric G-proteins are involved in signal transduction from cell surface receptors. There are several subfamilies of heterotrimeric G-proteins and that responsible for phosphoinositide metabolism is designated G_q. Trimeric G-proteins consist of a relatively large subunit (α), a slightly smaller one (β) and a much smaller one (γ). The G-protein is attached to only the cytoplasmic side of the plasma membrane, as represented in fig. 1.3.

In the resting state the three subunits are bound together and a molecule of GDP is bound to the α-subunit. On extracellular agonist activation, the receptor's conformation is altered such that it interacts with a nearby G-protein trimer. The interaction stimulates the dissociation of bound GDP from the α-subunit, which rapidly binds a molecule of GTP. The α-subunit-GTP complex dissociates from the $\beta\gamma$-complex and diffuses through the membrane until it encounters a molecule of phospholipase C (PLC; also referred to as phosphoinositidase C), which it activates. The bound GTP molecule is gradually hydrolysed to GDP, *i.e.* the α-subunit is a GTPase. The GDP-α-subunit complex does not activate PLC, dissociates from it and rejoins the $\beta\gamma$-complex, returning the G-protein to its resting state. G-proteins have been the subject of reviews.[3,19,20]

1.2.5 Phospholipase C

"Phospholipase C" is actually a family of related phosphodiesterases. These enzymes require calcium to function, and are membrane-bound proteins. They will hydrolyse PtdIns, PtdIns(4)P and PtdIns(4,5)P_2 *in vitro* but PtdIns(4,5)P_2 appears to be the main substrate *in vivo*.[21] The specific enzyme responsible for hydrolysing PtdIns(4,5)P_2 has been identified as phospholipase Cβ.[6] On activation by the GDP-α-subunit complex, this enzyme cleaves the bridging P–O bond as indicated in fig. 1.4 to generate Ins(1,4,5)P_3 and DAG.

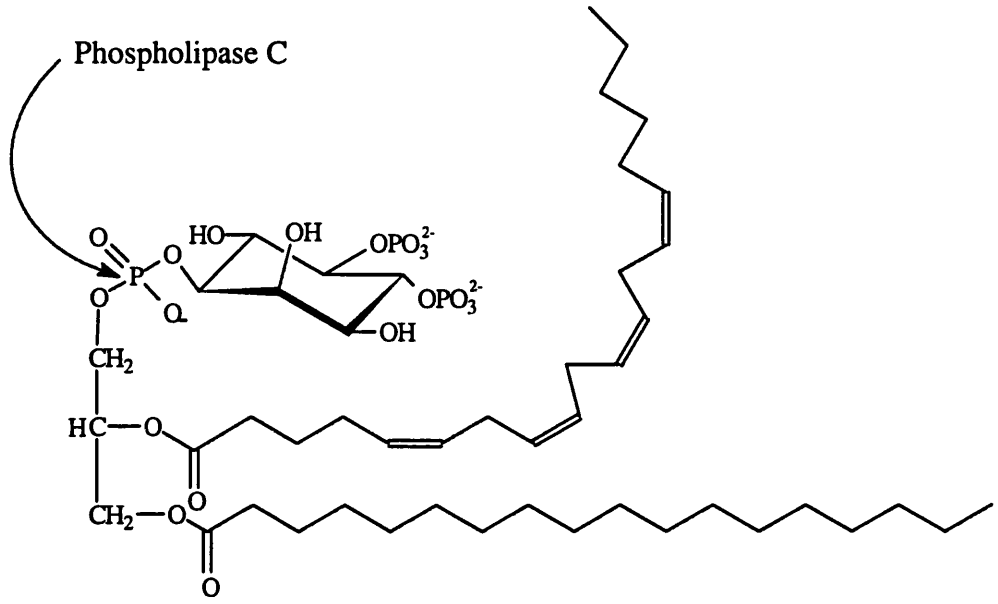


Fig. 1.4 Site of action of Phospholipase C

DAG itself is a second messenger. It stays in the plane of the membrane and activates protein kinase C.[7] This kinase phosphorylates specific serine and threonine residues in target proteins, including the insulin receptor. DAG can be subsequently cleaved to give arachidonic acid, the precursor to the eicosanoids. PLCβ is therefore an important catalyst for the liberation of two second messengers.

The highly charged Ins(1,4,5)P_3 molecule diffuses into the cytoplasm, crosses the 20nm gap separating the plasma membrane and the endoplasmic reticulum, and interacts with its receptor.

1.2.6 The Ins(1,4,5)P_3 Receptor

The Ins(1,4,5)P_3 receptor is situated in the lipid bilayer of the endoplasmic reticulum (ER). In cerebellar Purkinje cells, where it is expressed in abundance,[22] the receptor was found to be incorporated into the smooth ER, but was also present in rough ER and on the outer nuclear membrane.[11] Cloning of the receptor cDNA from several sources has confirmed that its structure is highly conserved and that it is a tetrameric ligand-gated ion channel, as represented in fig. 1.3. This Ca^{2+}-channel is dissimilar to Ca^{2+}-

channels in the plasma membrane,[11] but is both structurally and functionally similar to the ryanodine receptor, which is responsible for Ca^{2+} mobilisation in cardiac and skeletal muscle, and this similarity has been reviewed.[6]

Each of the four subunits of the Ins(1,4,5)P_3 receptor is capable of binding one molecule of Ins(1,4,5)P_3. Meyer *et al.*[23] found evidence of cooperativity and suggested that four molecules of Ins(1,4,5)P_3 had to bind sequentially for channel opening to occur, although other studies[24,25] found no cooperativity, suggesting that a single molecule of Ins(1,4,5)P_3 could open the channel. A recent study[26] using the purified type I receptor (*vide infra*) found positive cooperativity and evidence that kinetically the receptor has two states for Ca^{2+} release. One has a high affinity for Ins(1,4,5)P_3 and a low permeability to Ca^{2+} ions. This state causes a slow release of Ca^{2+} from the stores. The second has low affinity for Ins(1,4,5)P_3 and a high permeability to Ca^{2+} ions, and causes a rapid release of Ca^{2+}. Their data suggest that the fast phase of Ca^{2+}-release increases with increasing Ins(1,4,5)P_3 concentration, and that this receptor state is responsible for quantal Ca^{2+} release.[26] The discrepancies in cooperativity results[23–26] illustrate the difficulties biologists face in attempting to compare results from different experimental systems.

The receptor comprises three functional regions, the Ins(1,4,5)P_3-binding region, the Ca^{2+}-channel and a regulatory area.

i) ***Ins(1,4,5)P_3-binding region*** Ins(1,4,5)P_3 appears to bind within the amino-terminal 20% of the receptor, based upon photoaffinity labelling studies with [^{3}H](benzoyldihydrocinnamyl)aminopropyl-Ins(1,4,5)P_3[27] and mutagenesis studies.[28] Structure-activity relationships at the Ins(1,4,5)P_3 receptor are considered in detail in section 1.3.

ii) ***Ion channel region*** The 600 amino acids at the carboxyl terminus contain a hydrophobic transmembrane domain which is believed to constitute the Ca^{2+}-channel,[29] *i.e.* the Ca^{2+}-channel is an intrinsic part of the receptor. There appear to be 6–8 transmembrane units.[29–31]

iii) ***Regulatory region*** $Ins(1,4,5)P_3$-stimulated Ca^{2+}-release is an "all-or-nothing" effect,[6] but various processes can alter the effectiveness of $Ins(1,4,5)P_3$ on channel opening. The area of the receptor which is modified to effect these changes is located between the N-terminus $Ins(1,4,5)P_3$-binding region and the C-terminus channel. A cAMP-dependent protein kinase can phosphorylate two serine residues, although again there are conflicting reports of the effect. In some systems[32,33] a partial reduction of $Ins(1,4,5)P_3$-induced Ca^{2+}-release was found, while in others[34–36] Ca^{2+}-release was increased. At low concentrations ATP potentiates $Ins(1,4,5)P_3$-stimulated channel opening.[11] Ca^{2+} itself can exert a positive feedback[25] which has been reviewed.[6] A schematic representation of the $Ins(1,4,5)P_3$ receptor, summarising the points above, is given in fig. 1.5.

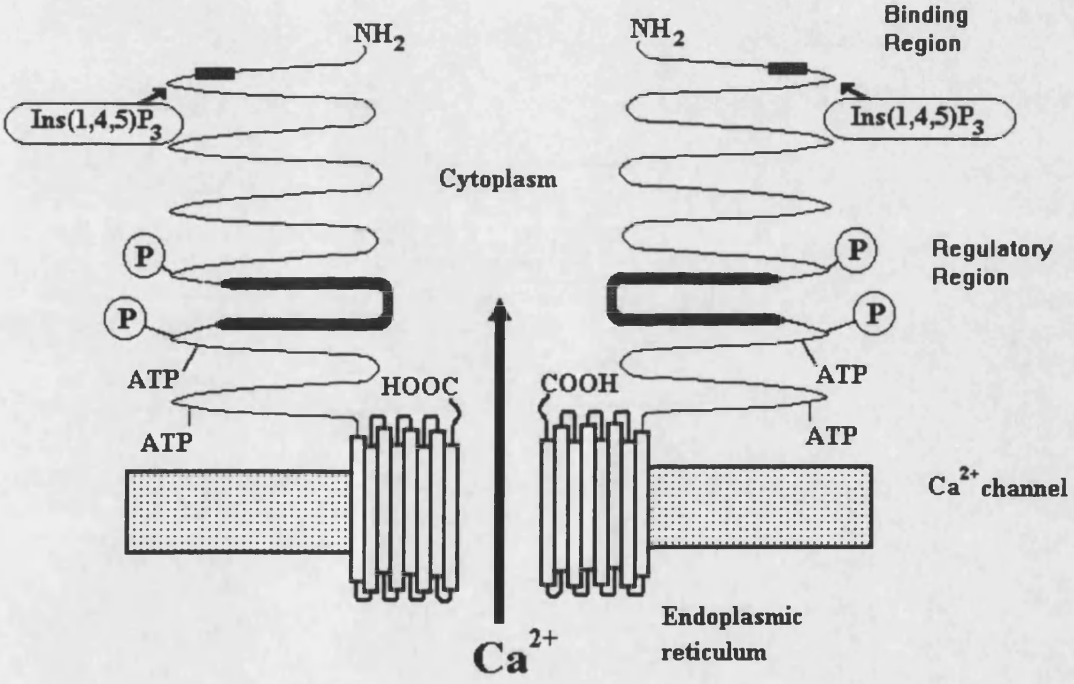


Fig.1.5 Schematic representation of the $Ins(1,4,5)P_3$ receptor showing the functionally distinct regions.

Subtypes of the $Ins(1,4,5)P_3$ receptor have been identified. It should be emphasised, however, that classification has not arisen on a classical pharmacological basis by

considering differing effects of compounds on different receptor populations (*e.g.* acetylcholine receptors stimulated by muscarine or nicotine). Rather, these proteins contain different primary sequences as identified by molecular cloning and sequencing experiments. (Early purification techniques[24] relied upon heparin-agarose columns, which, as heparin binds many receptor subtypes, provided a mixture of these subtypes).

In rat, type I, II and III receptors have been identified, containing 2749, 2701 and 2670 amino acids respectively and sharing 62–69% sequence homology.[29,37,38] De Smedt *et al.*[39] have shown that the type I receptor is present in most tissues, whereas types II and III are less widespread. Recently the human type I receptor has been purified and characterised from platelets.[40] Putative type IV receptors from mouse placenta[41] and rat basophilic leukaemic cells[39,42] and a putative type V receptor from mouse fibroblasts[39] have been reported.

It was originally believed that receptor subtypes were all homotetrameric. However, it has recently been shown that distinct monomer subunits combine to form heterotetramers in many cell types where two[43] or all[44] of receptor types I, II and III are abundantly expressed. This phenomenon could greatly increase the number of structurally distinct receptor types, although the observed proportions of heterotetramers suggest that they do not arise through random mixing.[43]

Any *physiological* significance of multiple receptor types remains to be established. Monkawa *et al.*[44] noted that in voltage-dependent K^+ channels and cyclic nucleotide-gated cation channels, hetero-oligomeric channels have different properties from the homo-oligomeric ones, and speculated that this could also be the case with the $Ins(1,4,5)P_3$ receptors.

Some experimental differences between receptor subtypes have been reported:

i) The protein kinase A phosphorylation sites (*vide ultra*) present in receptor type I are not present in types II and III.[43,44]

ii) There is a calmodulin binding-site in type I and II receptors that is not present in type III.[44] This may be significant as most of the effects of Ca^{2+} arise from its binding to calmodulin.

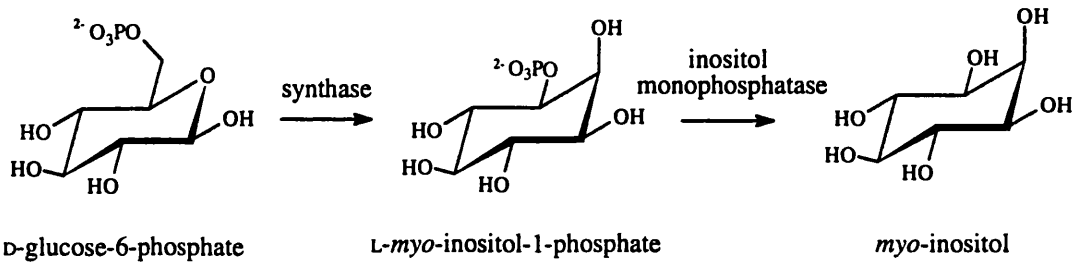
iii) Binding specificities of eight inositol phosphates were determined for the type I and type II receptors. The compounds displayed the same rank order, but different absolute values, the type II receptor having the higher affinity for Ins(1,4,5)P_3.[37] It has been suggested[37,44] that different receptor types may respond to different levels of Ins(1,4,5)P_3 within the cell. This is an attractive hypothesis because it represents a second mechanism (in addition to the dual affinity states observed in the type I receptor) for different amounts of Ca^{2+} to be released despite the observed "all-or-nothing" mechanism: different receptor subtypes would release their Ca^{2+} stores sequentially depending on their variable sensitivities to Ins(1,4,5)P_3.

iv) Wojcikiewicz[45] has demonstrated that type I, II and III receptors are down-regulated to different extents when AR4-2J cells are exposed to various extracellular agonists coupled to the Ins(1,4,5)P_3 second messenger pathway.

1.2.7 Metabolism of Ins(1,4,5)P_3

Anabolism: In mammals dietary intake is the main source of *myo*-inositol, which abounds in plant material. It can also be synthesised *de novo* from glucose-6-phosphate *via* L-Ins(1)P by the sequential action of the enzymes L-*myo*-inositol-1-phosphate synthase (see fig. 2.5, chapter two) and inositol monophosphatase (fig. 1.6). The latter enzyme also dephosphorylates Ins(4)P, Ins(5)P and Ins(6)P and is therefore an important component in the production of free inositol.

Lithium ions inhibit inositol monophosphatase and it has been suggested that this inhibition may contribute to the effectiveness of lithium salts in the treatment of manic depression.[46] As lithium has a notoriously low therapeutic index,[47] considerable effort has been invested in attempting to understand the mechanism of dephosphorylation and to prepare a less toxic inhibitor of inositol monophosphatase, and this work has been reviewed.[46]

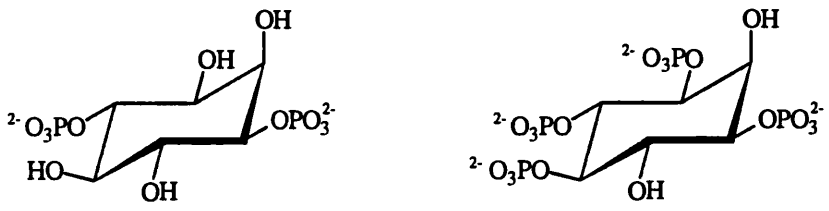


D-glucose-6-phosphate L-*myo*-inositol-1-phosphate *myo*-inositol

Fig. 1.6 *De novo* synthesis of *myo*-inositol

Inositol is incorporated into the phosphatidylinositol metabolic pathway by phosphatidylinositol synthetase, which combines it with cytidine-phosphatidate to form PtdIns (**7**) and cytidine 5'-monophosphate. The subsequent conversion of (**7**) into PtdIns(4,5)P_2 (**9**) was outlined in fig. 1.2.

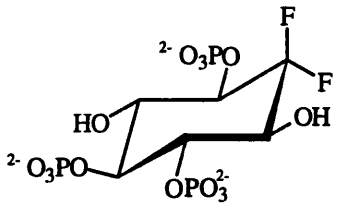
Catabolism: Once biological messengers have exerted their effects, they must be efficiently deactivated so the cell can return to its basal state. Ins(1,4,5)P_3 can be deactivated by a low affinity, high capacity 5-phosphatase which generates Ins(1,4)P_2 (**12**), or a high affinity, low capacity 3-kinase which produces Ins(1,3,4,5)P_4 (**13**). Dual deactivation systems with these affinity/capacity differences are found throughout nature, for example uptake-1 and uptake-2 of noradrenaline.[48] Both 5-phosphatase and 3-kinase have been purified and well characterised.[11]



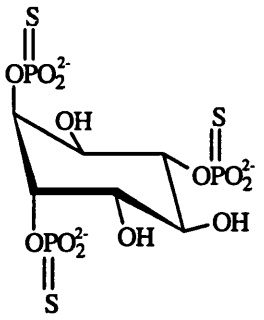
(12) **(13)**

Much effort has been invested in attempting to prepare suitable inhibitors of these enzymes to use as pharmacological tools (reviewed[11]). In brief, many analogues of Ins(1,4,5)P_3 bind to 5-phosphatase but few are substrates. Very few analogues bind to

3-kinase with high affinity and even fewer are substrates. Inhibitors of these enzymes which do not themselves mobilise intracellular Ca^{2+} are potentially useful and several have been developed, *e.g.* L-2-deoxy-2,2-difluoro-Ins(1,4,5)P_3 (**14b**) is a potent competitive inhibitor of 3-kinase[49] and L-*chiro*-Ins(1,4,6)PS_3 (**15**) potently inhibits 5-phosphatase.[50]



(14b)



(15)

$Ins(1,4)P_2$ has no known rôle in Ca^{2+} homeostasis, although it has been shown to allosterically activate 6-phosphofructo-1-kinase *in vitro*.[51] Whether $Ins(1,3,4,5)P_4$ has a physiological function is unclear and this controversy is reviewed in chapter two. Metabolites of $Ins(1,4,5)P_3$ themselves undergo extensive phosphorylation and dephosphorylation, resulting in a plethora of naturally occurring inositol phosphates (fig. 1.7), many of which do not have any known physiological significance. Inositol phosphate metabolism and potential biological functions of inositol phosphates other than $Ins(1,4,5)P_3$ have been reviewed in detail.[21,52]

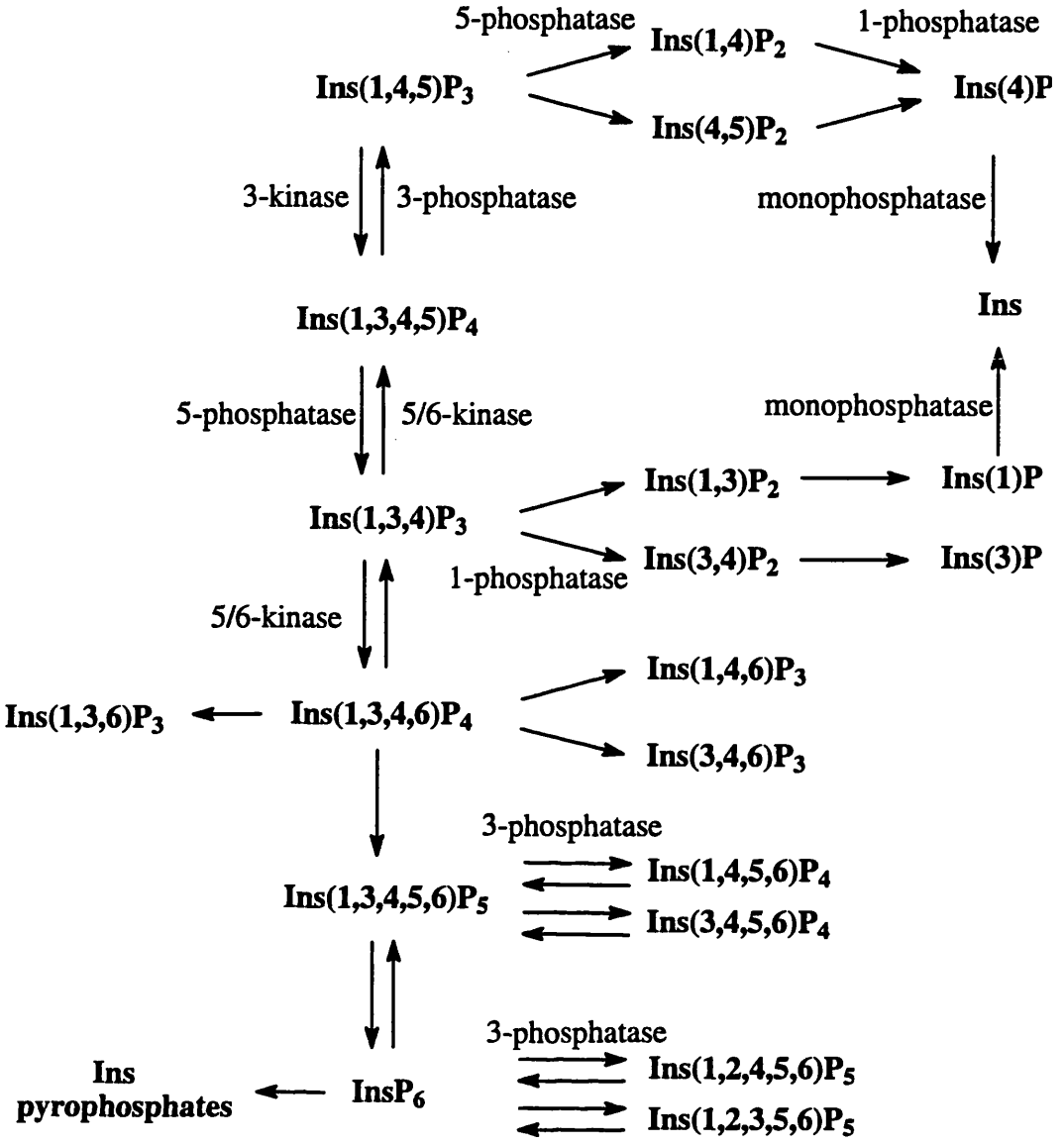


Fig. 1.7 Metabolism of inositol phosphates.

1.3 Structure-Activity Relationships at The $Ins(1,4,5)P_3$ Receptor

1.3.1 General Comments

An underlying aim of the work described in the subsequent chapters of this thesis was to ascertain whether various structurally diverse polyphosphates based on inositol, cyclopentane and glucose retained sufficient of the $Ins(1,4,5)P_3$ pharmacophore to mimic $Ins(1,4,5)P_3$-stimulated Ca^{2+}-mobilisation. A detailed understanding of structure-activity relationships at the $Ins(1,4,5)P_3$ receptor leading to a tentative definition of the pharmacophore is therefore essential. This subject has been recently reviewed[11] and will be considered in detail here.

The inositol polyphosphates described below have not all been tested in the same system. $Ins(1,4,5)P_3$ receptors from brain, *Xenopus* oocytes, Swiss 3T3 cells, GH_3 cells, hepatocytes, platelets, pancreatic and parotid acinar cells, skeletal muscle triads, SH-SY5Y neuroblastoma cells, rat basophilic leukaemic cells and *Limulus* photoreceptors are routinely used in pharmacological experiments. Therefore absolute values for half maximal concentration for calcium release (EC_{50}) and binding affinity are not directly comparable. However, similar patterns emerge from all systems and it is the rank order of potency that is important. (Rank orders of EC_{50} values are rarely different from rank orders of binding affinities; therefore, these two parameters are broadly interchangeable.) Where binding affinity or EC_{50} values are given, they are referenced to the value for $Ins(1,4,5)P_3$ from the same experiment whenever possible.

To date, no differences in rank order of affinity of a set of analogues tested in different purified receptor subtypes have been reported.

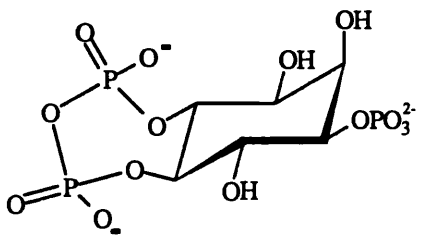
1.3.2 Agonists

Most active inositol phosphates and analogues examined to date are full agonists with respect to calcium mobilisation.

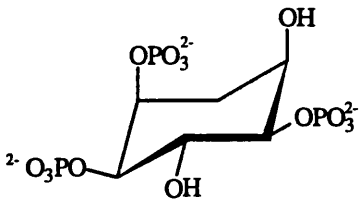
^{1}H, ^{13}C and ^{31}P NMR spectroscopic studies have established that the chair conformation of Ins(1,4,5)P_3 containing three equatorial phosphates and two equatorial hydroxyl groups is anancomeric at physiological pH.[53]

The Ins(1,4,5)P_3 receptor exhibits high stereoselectivity. In racemic mixtures, activity is usually attributable to one enantiomer, and eudismic ratios are high. This is exemplified by D- and L-Ins(1,4,5)P_3 (**3a** and **3b** respectively); (**3b**) does not mobilise Ca^{2+} to any significant extent and is 1000–2000 times more weakly bound than (**3a**).[54–56]

Irvine *et al.*[57] predicted that the minimum structural requirements for calcium release were *trans* vicinal phosphates on positions D-4 and D-5, and this is supported by the relative or absolute lack of activity of *inter alia*, *myo*-inositol,[58] Ins(1)P,[58] Ins(2)P,[58] DL-Ins(1,4)P_2,[57,58] DL-Ins(1,3,5)P_3,[54] L-Ins(1,4,5)P_3,[54–56] L-2-deoxy-2,2-difluoro-Ins(1,4,5)P_3 [L-2,2-F_2-Ins(1,4,5)P_3] (**14b**),[49] DL-*myo*-inositol 1-phosphate-4,5-pyrophosphate (**16**),[59] and derivatives in which all three phosphates have been replaced by sulphonamide, sulphate, methylphosphonate and carboxymethyl groups.[60] Additionally, D-3-deoxy-*muco*-Ins(1,4,5)P_3 (**17**), in which the position 4 phosphate is inverted, was found to bind almost 1000-fold more weakly to rat cerebellar membranes than Ins(1,4,5)P_3, compared to a 10-fold reduction by D-3-deoxy-Ins(1,4,5)P_3 (**18**).[61] It is worthy of note that replacement of phosphate groups with phosphorothioates gives compounds which often *do* release calcium (*vide infra*).



(16)

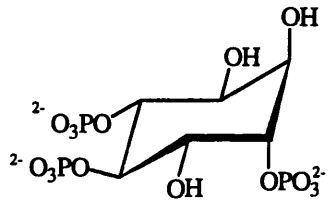


(17)

Each ring position of Ins(1,4,5)P_3 will now be considered independently.

Position 1: The presence of a phosphate group at position 1 of Ins(1,4,5)P_3 appears to be important in increasing the affinity between the molecule and its receptor as

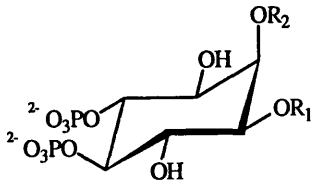
Ins(4,5)P_2 (**19**) is bound considerably less tightly than Ins(1,4,5)P_3.[58] Inversion of stereochemistry at position 1, to give D-*chiro*-Ins(1,3,4)P_3 (**20**) resulted in only a *ca.* 25-fold reduction in potency in permeabilised rat basophilic leukaemic cells [EC_{50} 4.2μM; *cf.* Ins(1,4,5)P_3 0.17μM].[62] The 1-phosphate group of Ins(1,4,5)P_3 has been extensively modified with little effect on potency. Henne *et al.*[63] attached various large substituents to this site and found that even D-*N*-octylaminoethanol(1)1-phospho-*myo*-inositol 4,5-bisphosphate (**21**) was only about 7-fold less potent than Ins(1,4,5)P_3 at mobilising calcium from guinea pig parotid acinar cells. Other groups have taken advantage of this fact to attach photoaffinity ligands and fluorescent labels to this site.[64,65]



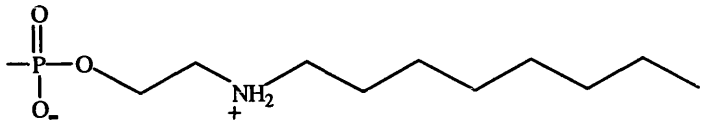
(**20**)

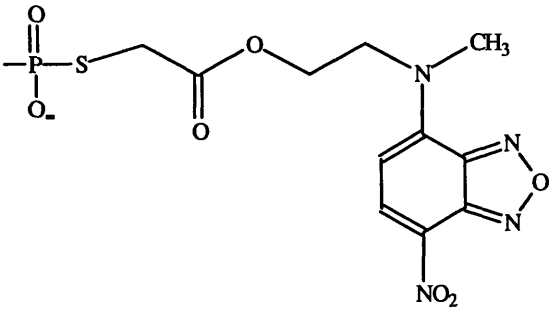
Potter and co-workers[64] prepared a nitrobenzoxdiazole derivative (**22**) from DL-Ins(1,4,5)P_3–1S (**23**), while Prestwich *et al.*[65] prepared racemic radiolabelled (azidosalicyl)aminopropyl (**24**) and (benzoyldihydrocinnamyl)aminopropyl (**25**) derivatives. All were found to be potent calcium mobilisers (EC_{50} for **22** 0.64μM; *cf.* Ins(1,4,5)P_3 0.08μM),[66] or to display high affinity for the receptor (K_i for **24** and **25** 0.236μM and 0.500μM respectively; *cf.* Ins(1,4,5)P_3 0.01μM).[27]

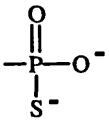
Given the above observations, it is reasonable to suggest that the 1-phosphate group undergoes an electrostatic interaction with a cationic residue (arginine or lysine) such that the molecule is tightly bound to the receptor in an orientation allowing protrusion of the bulky substituents away from the receptor. Ins(2,4,5)P_3 (**26**) is only about five times less potent than Ins(1,4,5)P_3.[57] It may tentatively be proposed that transfer of the phosphate from position 1 to position 2 does not completely abolish interaction with this site.

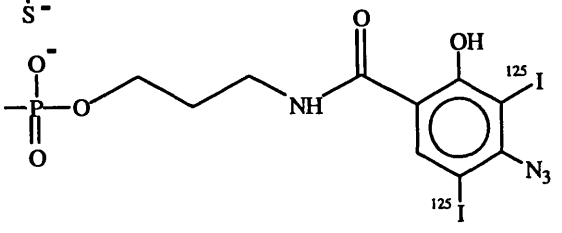


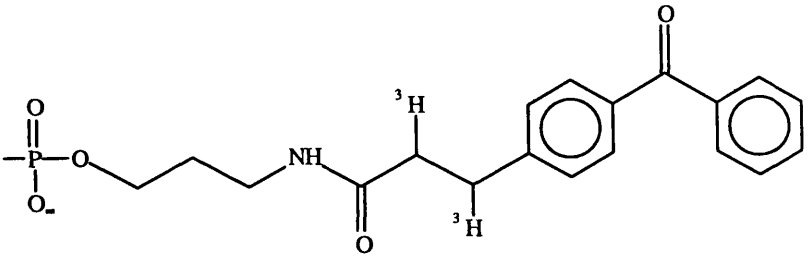
(19) $R_1=R_2=H$

(21) $R_1=$  ; $R_2=H$

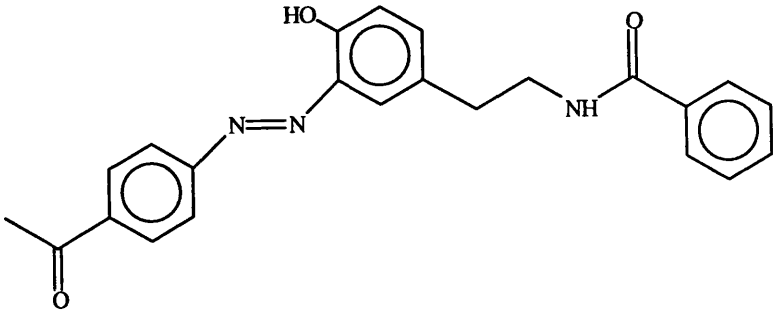
(22) $R_1=$  ; $R_2=H$

(23) $R_1=$  ; $R_2=H$

(24) $R_1=$  ; $R_2=H$

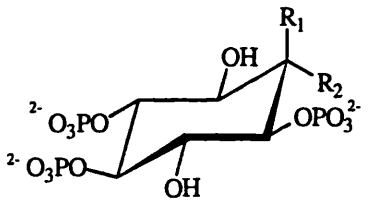
(25) $R_1=$  ; $R_2=H$

(26) $R_1=H$; $R_2=PO_3^{2-}$

(27) $R_1=PO_3^{2-}$; $R_2=$ 

(28) $R_1=R_2=PO_3^{2-}$

Position 2: Hirata and co-workers[67,68] have prepared many analogues of Ins(1,4,5)P_3 modified at position 2. They found that even incorporation of bulky substituents had little effect on calcium-releasing activity. For example, racemic (**27**) had an EC_{50} value of 0.3μM compared to 0.2μM for Ins(1,4,5)P_3 in permeabilised guinea pig peritoneal macrophages.[67] Incorporation of an additional phosphate group at position 2 to give Ins(1,2,4,5)P_4 (**28**) has produced a potent calcium-releasing inositol tetrakisphosphate,[69,70] which exhibited about half the potency of Ins(1,4,5)P_3 in SH-SY5Y neuroblastoma cells [EC_{50} 106nM; *cf.* Ins(1,4,5)P_3 51.6 nM].[70] Replacement of the position 3 hydroxyl group with a fluorine atom did not significantly alter Ca^{2+}-mobilising potency.[70]

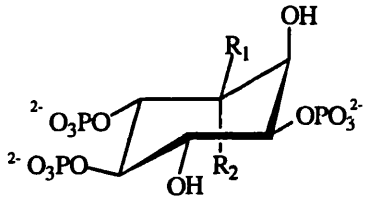


(**14**) R_1=R_2=F (**a**=D; **b**=L)
(**28**) R_1=OPO_3^{2-}; R_2=H
(**29**) R_1=R_2=H
(**30**) R_1=H; R_2=OH
(**31**) R_1=F; R_2=H
(**32**) R_1=H; R_2=F

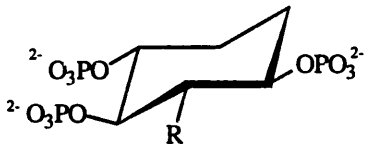
The fact that both hydroxyl deletion[67] and inversion[71] at position 2 (to give DL-2-deoxy-Ins(1,4,5)P_3 [**29**] and DL-*scyllo*-Ins(1,2,4)P_3 [**30**] respectively) only result in an approximately 2.5-fold decrease in calcium mobilising activity suggests that the 2-hydroxyl group has only a weak interaction with the receptor.

Studies involving 2-fluorinated derivatives have helped probe the nature of this interaction. (The reasonable assumption is made that L-enantiomers of all the following analogues are inactive, as was demonstrated for DL-2,2-F_2-Ins(1,4,5)P_3 [**14ab**]).[49] 2-Deoxy-2-fluoro-Ins(1,4,5)P_3 (**31**) was found to be equipotent with Ins(1,4,5)P_3 in releasing calcium in SH-SY5Y cells [DL-**31** EC_{50} 104.7nM; *cf.* Ins(1,4,5)P_3 51.6nM],[72] whereas 2F-*scyllo*-Ins(1,4,5)P_3 (**32**) was about 3-fold less potent [DL-**32** EC_{50} 0.77μM; *cf.* Ins(1,4,5)P_3 0.13μM].[73] D-2,2-F_2-Ins(1,4,5)P_3 (**14a**) was about 1.5 times less potent [EC_{50} 0.21μM; *cf.* Ins(1,4,5)P_3 0.13μM].[49] Both (**31**) and (**14a**) can accept, but not donate, hydrogen bonds;[74] (**32**) can donate, but not accept hydrogen bonds. Thus, the 2-hydroxyl group of Ins(1,4,5)P_3 possibly accepts a hydrogen bond from its receptor.

Position 3: Modifications of position 3 of Ins(1,4,5)P_3 have included its hydroxyl deletion,[75] inversion[76,77] and isosteric replacement with fluorine[75,76] (to give **18**, **33** and **34** respectively). All these changes yielded relatively potent calcium mobilisers (*e.g.* EC_{50} of **33** 1.00μM; *cf.* Ins(1,4,5)P_3 0.1μM in SH-SY5Y cells).[78] The chiral 3-*C*-trifluoromethyl analogue (**35**) was only threefold less potent than Ins(1,4,5)P_3 in binding assays,[79] suggesting that the receptor can tolerate some steric bulk in the axial position 3 region of Ins(1,4,5)P_3 even when the 3-hydroxyl group is retained. D-2,3-Dideoxy-Ins(1,4,5)P_3 (**36**), in which hydroxyl groups at positions 2 and 3 have been deleted, still binds firmly and is a relatively potent calcium releasing analogue.[75] These results suggest that, like position 2, position 3 has little importance in receptor recognition and calcium release.



(**18**) R_1=R_2=H
(**33**) R_1=H; R_2=OH
(**34**) R_1=F; R_2=H
(**35**) R_1=OH; R_2=CF_3
(**37**) R_1=Cl; R_2=H
(**38**) R_1=Br; R_2=H
(**39**) R_1=OCH_3; R_2=H
(**40**) R_1=$OCH_2CO_2^-$; R_2=H



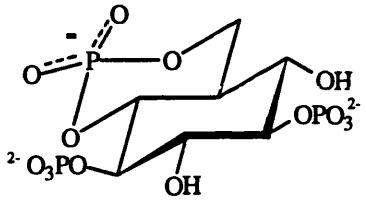
(**36**) R=OH
(**48**) R=H

It has been demonstrated, however, that this position may have poor tolerance for bulky groups. A smooth increase in EC_{50} values (Ca^{2+}-mobilisation in SH-SY5Y neuroblastoma cells) was observed when replacing the position 3 hydroxyl of Ins(1,4,5)P_3 (EC_{50} 0.05μM) with H (**18**; 0.16μM), F (**34**; 0.12μM), Cl (**37**; 0.64μM), Br (**38**; 1.1μM) and methoxy (**39**; 7.7μM),[80] and replacement with ethoxy or *n*-propoxy resulted in even larger increases.[81] Notably, DL-3-*O*-methylenecarboxylate-Ins(1,4,5)P_3

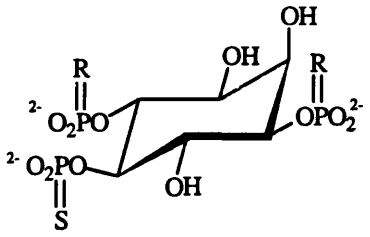
(**40**) was more potent than this series would predict (EC_{50} 3.5μM),[82] possibly because of the charge on the carboxylate.[81]

Whether Ins(1,3,4,5)P_4 can mobilise Ca^{2+} *via* the Ins(1,4,5)P_3 receptor is disputed, and this controversy is reviewed in chapter two.

Position 4: No analogues of Ins(1,4,5)P_3 modified exclusively at position 4 have yet been described (see above for inversion of this position in 3-deoxy analogues), but a derivative in which the position 4 phosphate has been conformationally restricted (with concomitant charge reduction) has recently been described.[83] Racemic (**41**) (note also that the hydroxyl group at position 2 is inverted) was reported to possess an EC_{50} value *ca.* 40-fold higher than that of Ins(1,4,5)P_3.[83]



(**41**)

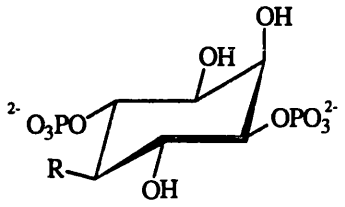


(**42**) R=O
(**43**) R=S

Position 5: Potentiometric studies have shown that increase in specific binding to brain membranes as a function of pH coincides with increasing ionisation of Ins(1,4,5)P_3,[84] and particularly of the position 5 phosphate.[85] This suggests that the position 5 phosphate is important in the binding process.

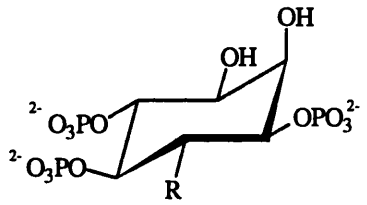
Substitution of the phosphate group at position 5 with a phosphorothioate as in DL-Ins(1,4,5)P_3–5S (**42**), produced a potent agonist which could not be deactivated by 5-phosphatase.[86] This compound was more potent than the multiply modified DL-Ins(1,4,5)PS_3 (**43**) (EC_{50} values of 0.8μM and 2.5μM respectively; *cf.* Ins(1,4,5)P_3 0.11μM),[86] which is not surprising as it more closely resembles the natural ligand.

A chiral 5-methylenephosphonate analogue (**44**) of Ins(1,4,5)P_3 has been synthesised and has been shown to be another long-lived agonist of calcium release.[87] A 5-phosphonate analogue (**45**) has been reported to be an antagonist of calcium release;[88] the corresponding difluorinated phosphonate (**46**) was inactive, however.[88]



(**44**) R=$CH_2PO_3^{2-}$
(**45**) R=OPO_2^- CH_3
(**46**) R=OPO_2^- CHF_2

Position 6: The hydroxyl group at position 6 is the most sensitive of the three unphosphorylated hydroxyl groups in the cyclitol to modification. D-6-Deoxy-Ins(1,4,5)P_3 (**47**) was found to be 400-fold less potent than Ins(1,4,5)P_3 at binding to the receptor[78] and 70-fold less potent at releasing calcium.[89] The receptor binding potency is comparable with that of D-2,3,6-trideoxy-Ins(1,4,5)P_3 (**48**), which was found to be about 600-fold weaker than Ins(1,4,5)P_3.[75] Comparing the relative potencies of (**48**) and (**36**) (above) indicates that loss of binding and calcium releasing potencies of (**48**) is almost exclusively due to the absence of the 6-hydroxyl group.



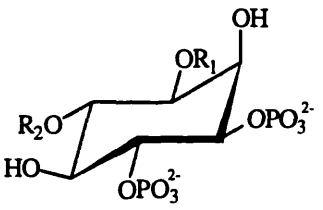
(**47**) R=H
(**49**) R=OCH_3
(**50**) R=OPO_3^{2-}
(**51**) R=F
(**52**) R=CH_3

It has been suggested that the 6-hydroxyl group may donate a hydrogen bond to an amino acid residue in the receptor,[75] or that intramolecular hydrogen bonding to the 1- or 5-phosphate groups may be important in defining the receptor-recognising properties of Ins(1,4,5)P_3.[90]

The receptor probably cannot tolerate steric bulk at position 6. DL-6-*O*-Methyl-Ins(1,4,5)P_3 (**49**) is 120-fold less potent than Ins(1,4,5)P_3 (ref.54) and Ins(1,4,5,6)P_4 (**50**) is inactive.[80]

Although Ley and co-workers have prepared DL-6-deoxy-6-fluoro-Ins(1,4,5)P_3 (**51**) and DL-6-deoxy-6-methyl-Ins(1,4,5)P_3 (**52**) from benzene,[91,92] biological data on these compounds have not been reported.

Multiple modifications: Ins(1,3,4,6)P_4 (**53**) is formed naturally and was first identified in adrenal glomerulosa cells exposed to angiotensin II.[93,94] It is approximately ten times less potent than Ins(1,4,5)P_3 at mobilising intracellular calcium,[95,96] and has been unambiguously demonstrated to compete for the same receptor site and to release calcium from the same store as Ins(1,4,5)P_3.[95] Ins(1,3,4,6)P_4 only releases 80–90% of the Ca^{2+} mobilised by Ins(1,4,5)P_3 in SH-SY5Y neuroblastoma cells,[95,97] *i.e.* it is a partial agonist (see section 1.3.3).

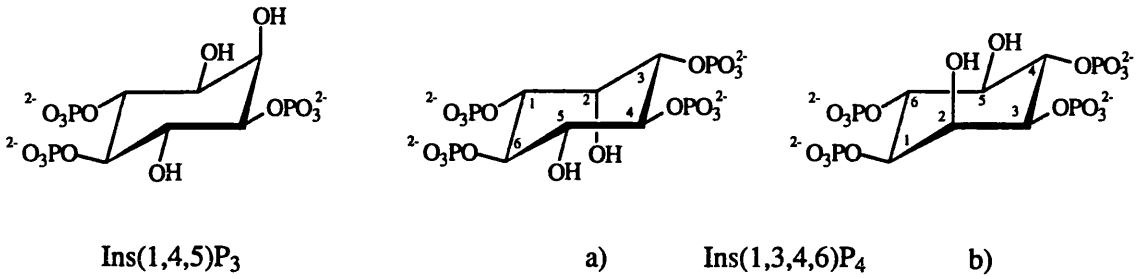


(**53**) R_1=R_2=PO_3^{2-}
(**54**) R_1=H; R_2=PO_3^{2-}
(**55**) R_1=PO_3^{2-}; R_2=H

It is interesting that (**53**) should have any calcium-releasing capacity at all, since the molecule lacks a 4,5-bisphosphate arrangement. However, two binding orientations can be envisaged for (**53**) such that a pseudo D-4, D-5- (or, more precisely, D-*threo*-) bisphosphate moiety is presented to the receptor, and these are illustrated in fig.1.8.

In arrangement (a; fig. 1.8), position 5 of Ins(1,3,4,6)P_4 mimics position 6 of Ins(1,4,5)P_3 with retention of equatorial displacement of the hydroxyl group; in orientation b), position 2 of Ins(1,3,4,6)P_4 becomes pseudo-position 6 and the hydroxyl group is inverted. As stated above, position 6 of Ins(1,4,5)P_3 is sensitive to change. Therefore, conformation a) is intuitively more likely to be that responsible for Ca^{2+}

mobilisation. Two related enantiomeric pairs of trisphosphates have been prepared and tested, namely D- (**54a**) and L-Ins(1,4,6)P_3 (**54b**), and D- (**55a**) and L-Ins(1,3,4)P_3 (**55b**).



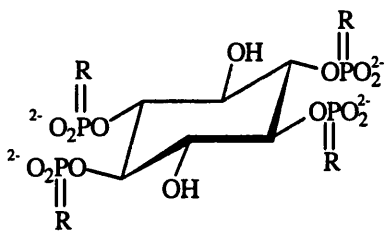
Ins(1,4,5)P_3 a) Ins(1,3,4,6)P_4 b)

Fig. 1.8 Possible binding orientations of Ins(1,3,4,6)P_4

In permeabilised rabbit platelets, (**54a**) and (**55b**) were full agonists for Ca^{2+}-release [EC_{50} 1.56 and 8.05μM respectively; *cf.* Ins(1,4,5)P_3 0.69μM], while (**54b**) and (**55a**) only mobilised Ca^{2+} at concentrations above 100μM.[98] Compounds (**54a**) and (**55b**) were also full agonists in rat basophilic leukaemic cells.[99] In *Limulus* photoreceptors, which tend to show an "all-or-nothing" response to agonists, (**55b**) produced a rapid burst of depolarisation similar to Ins(1,4,5)P_3, while (**55a**) did not.[100]

Therefore, eutomers in both cases were the enantiomers which contained a D-*threo* bisphosphate arrangement. Similarly to (**53**), two binding orientations can be proposed for each of these eutomers, such that the bisphosphate mimics that of Ins(1,4,5)P_3, and these are presented in fig. 1.9.

scyllo-Ins(1,2,4,5)P_4 (**56**), in which the position 2 hydroxyl of Ins(1,4,5)P_3 is inverted and phosphorylated, released Ca^{2+} with equal potency to Ins(1,4,5)P_3.[72] This is interesting as (**56**) is a symmetrical compound with only one possible binding orientation.



(**56**) R=O
(**57**) R=S

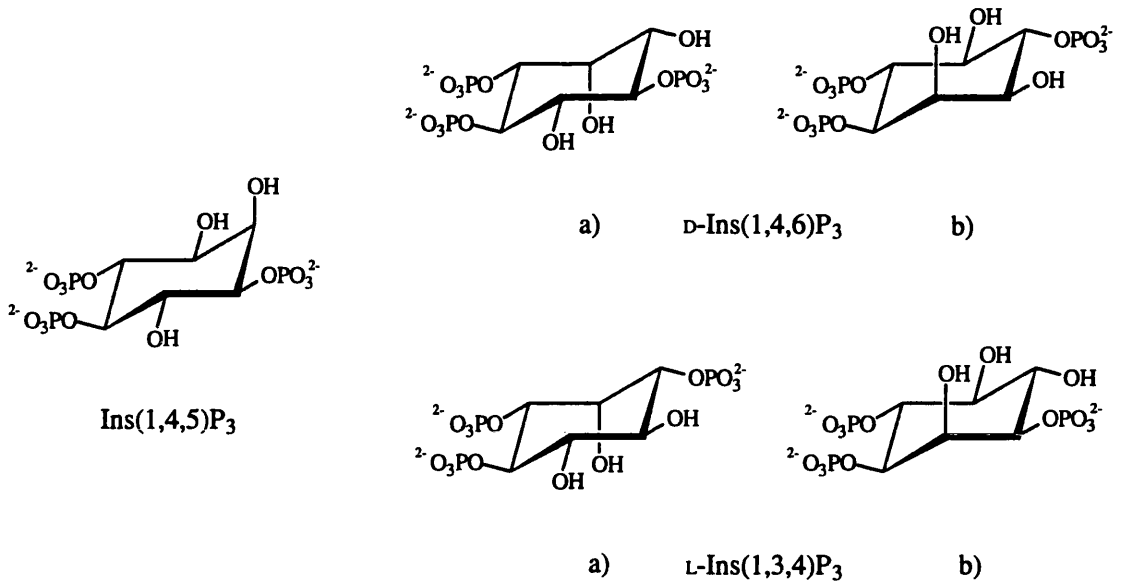


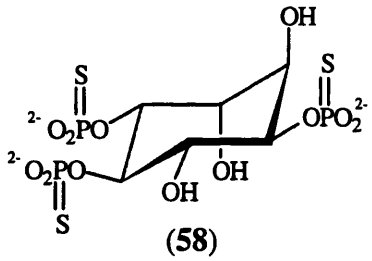
Fig. 1.9 Possible binding orientations of D-Ins(1,4,6)P_3 and L-Ins(1,3,4)P_3

The most potent agonists described to date are the naturally occurring adenophostins, which are discussed in chapter five.

In conclusion, a reassessment of requirements for agonism and definition of the pharmacophore would suggest a D-*threo* bisphosphate (or phosphorothioate) arrangement to be an essential feature, while a third suitably orientated phosphate and a pseudo-position 6 hydroxyl group increase potency.

1.3.3 Partial Agonists

As discussed above, Ins(1,3,4,6)P_4 was the first partial agonist to be described, releasing 80–90% of the Ca^{2+} mobilised by Ins(1,4,5)P_3 in SH-SY5Y cells. *scyllo*-Ins(1,2,4,5)PS_4 (**57**) exhibited similar properties.[72]



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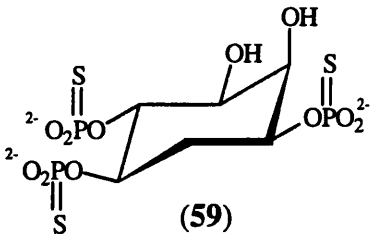

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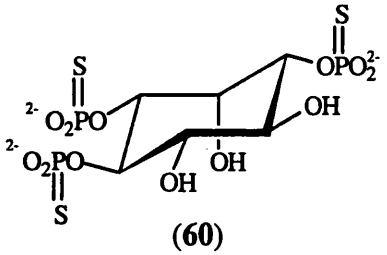

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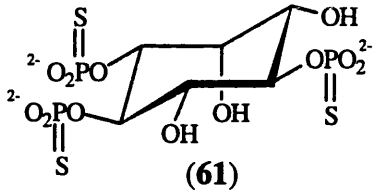
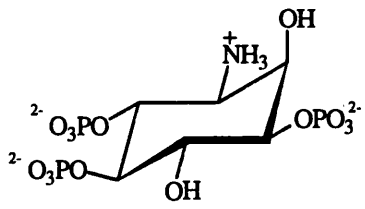


(61)

Only a few other partial agonists have been identified. L-*chiro*-Ins(2,3,5)PS_3 (**58**) and D-6-deoxy-Ins(1,4,5)PS_3 (**59**) both competed fully with Ins(1,4,5)P_3 for [^{3}H]Ins(1,4,5)P_3 binding sites on adrenal cortical membranes, but, respectively, released 34% and 42% of the calcium released by Ins(1,4,5)P_3.[97] DL-Ins(1,3,4)PS_3 (**60**) and DL-Ins(1,4,6)PS_3 (**61**) were also found to be partial agonists, releasing *ca.* 30% and 20% respectively of the Ca^{2+} store mobilised by Ins(1,4,5)P_3.[101] The partial agonism of these trisphosphorothioates is interesting as the corresponding trisphosphates are all full agonists.[77,89,98,100,102]



(62)

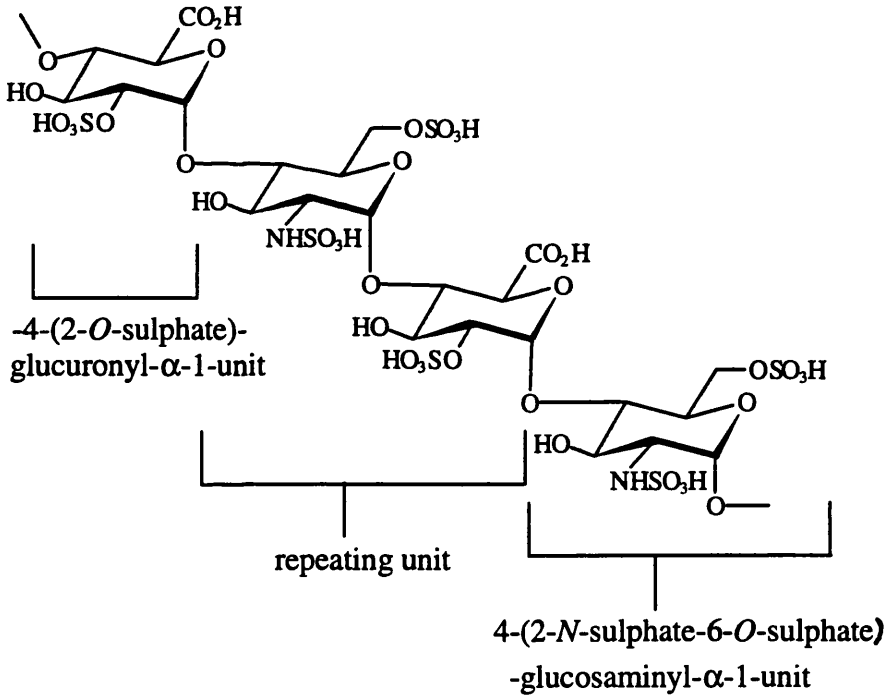
D-3-Amino-3-deoxy-Ins(1,4,5)P_3 (**62**) has been characterised as a pH-dependent partial agonist.[103] Thus, at pH 7.2 and 7.6 (**62**) was a full agonist [EC_{50} values 1.07 and 1.49μM respectively; *cf.* Ins(1,4,5)P_3 0.05 and 0.11μM respectively], while at pH 6.8 it was a partial agonist, releasing *ca.* 80% of the Ca^{2+} store mobilised by Ins(1,4,5)P_3.[103] This

was surprising as the amino function would be expected to be in its conjugate acid form at all three pH values. It has been suggested[103] that the lower pH might cause conformational changes in amino acid residues around the binding site.

Too few partial agonists have been prepared to allow generalisations to be made as to structural requirements for partial agonism. This is a challenge of the next few years.

1.3.4 Antagonists

The most well characterised antagonism of Ins(1,4,5)P_3-induced Ca^{2+} release is produced by heparin (**63**). The competitive and reversible inhibitory action of heparin has been demonstrated to be mediated through its interaction with the Ins(1,4,5)P_3 receptor in several cell types,[104–108] and it has been shown that both *N*- and *O*-linked sulphate groups are important.[109] Ghosh *et al.*[105] calculated a K_i value of 2.7nM for 4–6kDa heparin in purified microsomal membranes, making it a potent antagonist.

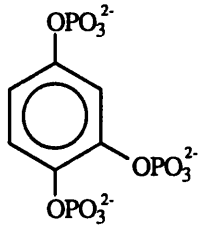


The basic structure of heparin (**63**)

Heparin is of little use in structure-activity studies as it is a large molecule and relatively non-selective, for example it also inhibits the ability of Ins(1,3,4,5)P_4 to bind to cerebellar membranes.[110] However, the molecule has proved useful in other experiments. Showing that the activity of novel agonists is heparin-sensitive demonstrates that they compete for the same receptor site as Ins(1,4,5)P_3. And, when isolating the Ins(1,4,5)P_3 receptor from rat brain, Supattapone *et al.*[24] used a heparin-agarose column to purify the receptor.

Another antagonist is the polyoxoanion decavanadate, which inhibited Ins(1,4,5)P_3-induced calcium release in permeabilised rat insulinoma and PC12 cells,[111] but the agent was subsequently found to possess low specificity, binding to all known recognition sites for both Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4.[112] Strupish *et al.*[112] suggested that oxygen atoms become placed in areas usually occupied by the 4,5-bisphosphate moiety of Ins(1,4,5)P_3.

There have been two reports of small-molecule antagonists of calcium release by Ins(1,4,5)P_3. The first was the 5-phosphonate analogue (**45**) of Ins(1,4,5)P_3,[88] the biological data for which have only appeared in preliminary form and have yet to be confirmed. The second was benzene-1,2,4-trisphosphate (**64**),[113] which only grossly resembles Ins(1,4,5)P_3 insomuch as having vicinal phosphates on one side of the molecule and a third phosphate on the other side. Compound (**64**) was reported to inhibit Ins(1,4,5)P_3-induced calcium mobilisation in a dose-dependent manner.[113]



(64)

1.3.5 Summary

A model of the binding site of the Ins(1,4,5)P_3 receptor may be tentatively proposed from the biological results of the compounds discussed in section 1.3. The model is schematically represented in fig.1.10.

1.4 Synthesis of Inositol Phosphates from *myo*-Inositol

1.4.1 General Considerations

The study of structure-activity relationships at the Ins(1,4,5)P_3 receptor (and other binding proteins) requires syntheses of analogues. The most common starting material is *myo*-inositol (although many other imaginative compounds have been used[11]) and a number of problems must be overcome:

i) Selective protection of some of the hydroxyl groups must be achieved to provide the phosphorylation precursor.

ii) Selective protection of the *meso*-starting material usually leads to racemic mixtures of subsequent compounds. Occasionally, one enantiomer may have an unpredicted or unwanted activity (*e.g.* **14b** inhibited the phosphorylation and dephosphorylation of its enantiomer **14a**). Therefore, ideally intermediates should be resolved to provide optically pure target compounds.

iii) A phosphorylation procedure must be employed such that cyclic phosphates do not form.

iv) The protected, phosphorylated intermediate must be deprotected without phosphate migration and the final polyphosphate must be efficiently purified.

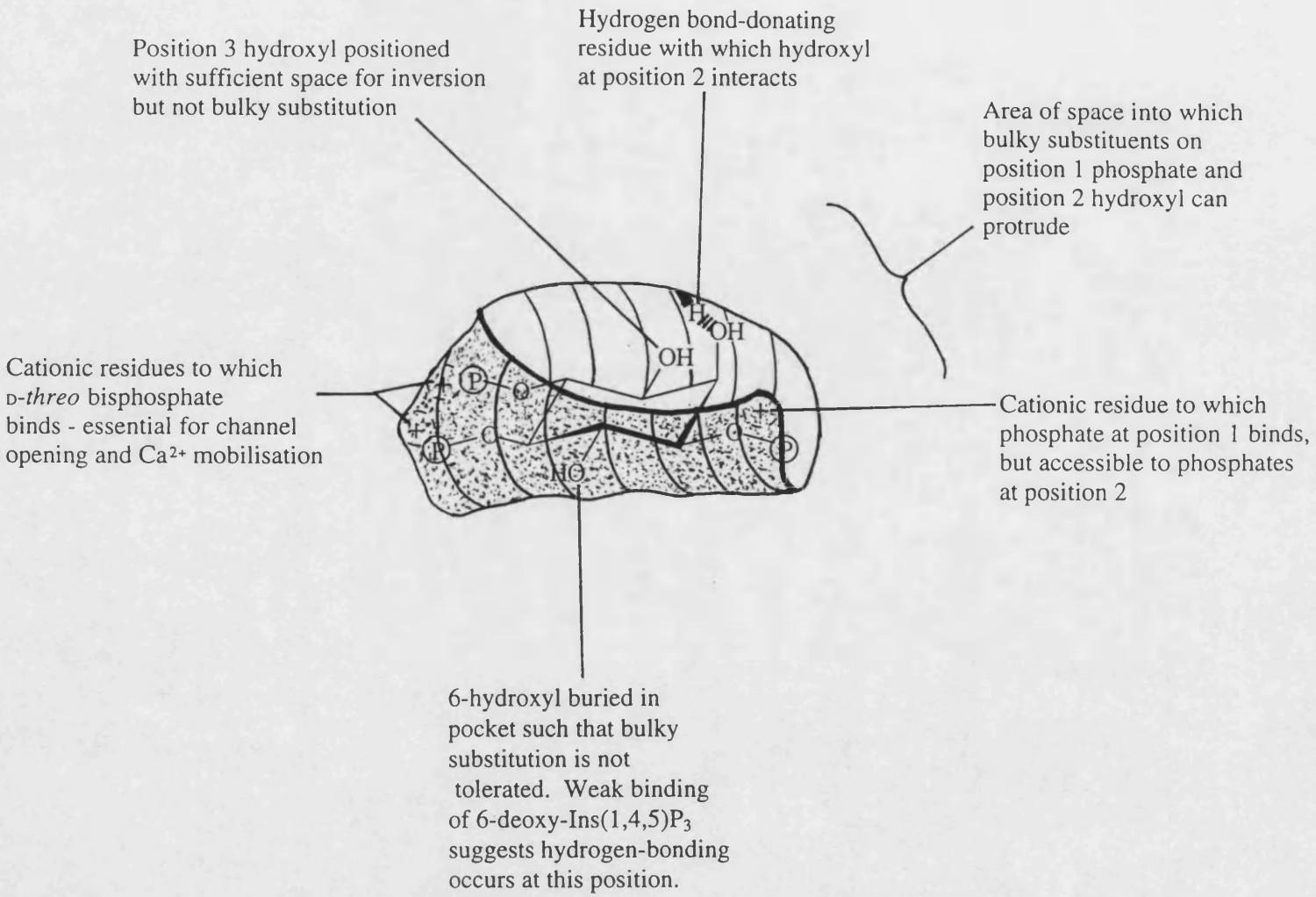


Fig. 1.10 Schematic representation of the proposed shape of the Ins(1,4,5)P_3 binding site.

The discovery of the second messenger function of Ins(1,4,5)P_3 in 1983[18] led to considerable chemical investigation of the synthesis of inositol phosphates. During the late 1980s, as chemists attempted to solve the problems outlined above, the inositol phosphates they synthesised seemed almost chosen at random. More recently, choices of target compound have become more rational, in an effort to probe structure-activity relationships, as described in section 1.3.

Detailed reviews of inositol phosphate chemistry and the preparation of inositol phosphates have appeared.[11,114–116] Additionally, two excellent complementary texts on protecting group chemistry have been published.[117,118] Here, the subject will be covered by reviewing a single typical example. The synthesis of D- (**55a**) and L-*myo*-inositol-1,3,4-trisphosphate (**55b**) by Riley *et al.*[100] illustrates many of the difficulties described above.

1.4.2 Synthesis of D- and L-*myo*-inositol-1,3,4-trisphosphate

D-Ins(1,3,4)P_3 is produced *in vivo* by the action of 5-phosphatase on Ins(1,3,4,5)P_4 and occupies a central position in inositol phosphate metabolism (reviewed[52,100]). There had been conflicting reports in the literature with respect to a) the absolute configuration of synthetic enantiomers of Ins(1,3,4)P_3 and b) its biological properties, particularly with respect to Ca^{2+} mobilisation *via* the Ins(1,4,5)P_3 receptor. Consequently, Riley *et al.*[100] designed a novel route to these enantiomers.

The triols required for phosphorylation were D- (**65b**; fig. 1.11) and L-2,4,5-tri-*O*-benzyl-*myo*-inositol (**65a**), and so a route to place benzyl ethers in these three positions was required. Treatment of *myo*-inositol with 2,2-dimethoxypropane and PTSA followed by benzoyl chloride allowed the isolation of the poorly soluble DL-3,6-di-*O*-benzoyl-1,2:4,5-di-*O*-isopropylidene-*myo*-inositol (**66**).[119] Cleavage of benzoates under basic conditions followed by allylation with sodium hydride and allyl bromide provided DL-3,6-di-*O*-allyl-1,2:4,5-di-*O*-isopropylidene-*myo*-inositol (**67**). Acidic hydrolysis afforded tetrol (**68**) and stannylene-mediated *p*-methoxybenzylation gave (**69**). Benzylation with sodium hydride and benzyl bromide provided fully protected DL-1,4-

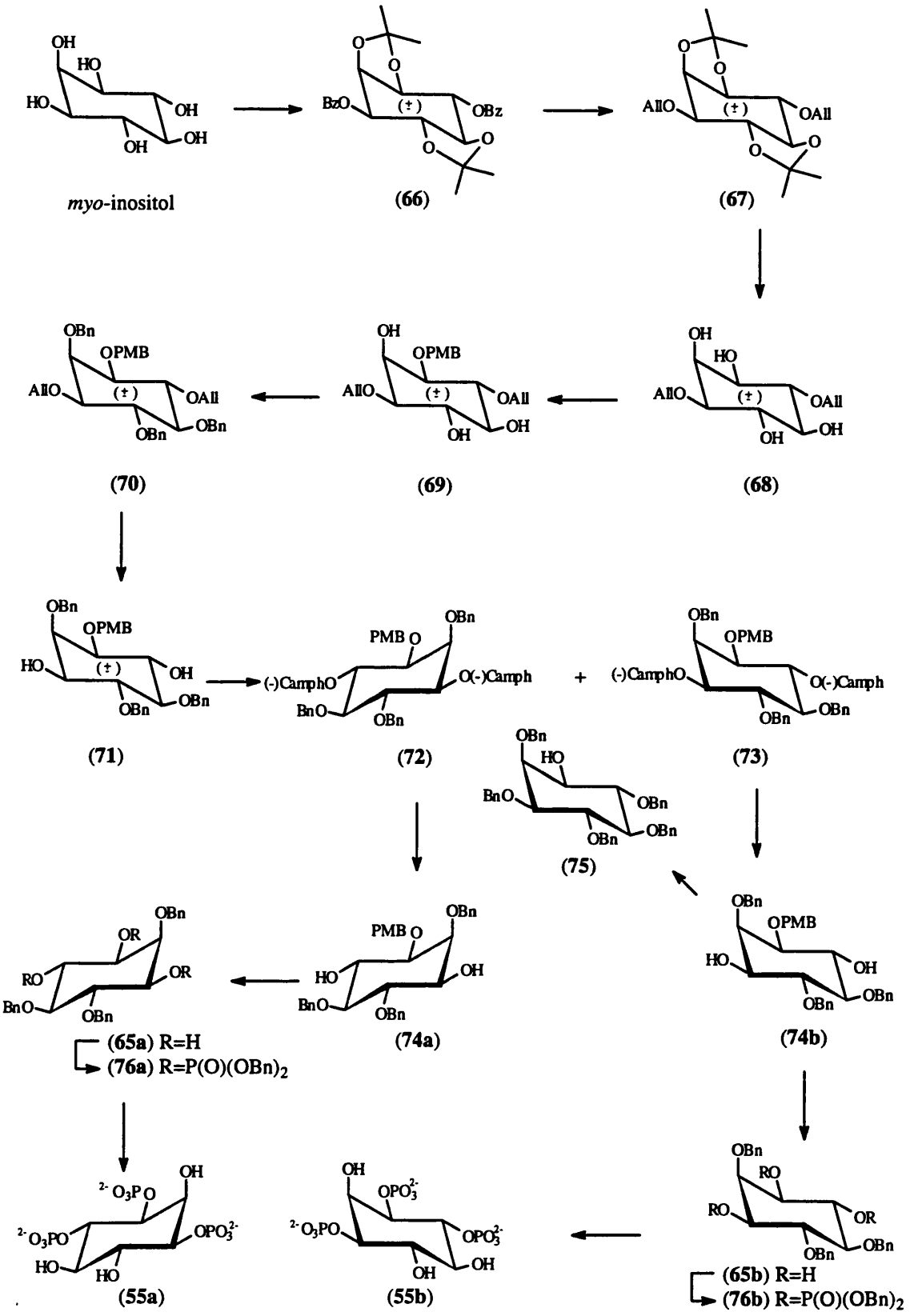


Fig. 1.11 Synthesis of the enantiomers of Ins(1,3,4)P_3.

di-*O*-allyl-2,5,6-tri-*O*-benzyl-3-*O*-(*p*-methoxybenzyl)-*myo*-inositol (**70**). Both allyl and *p*-methoxybenzyl ethers may be removed in the presence of benzyl ethers so the six steps so far, employing acetals, esters and three varieties of ether had provided appropriate regiochemical protection, but intermediate (**70**) was still racemic. Therefore, resolution was required.

Isomerisation of the allyl ethers to prop-1-enyl ethers followed by hydrolysis with mild acid, provided racemic diol (**71**). On esterification with 2 equiv. of the optically pure (*S*)-(–)-ω-camphanic chloride, the enantiomers of (**71**) were converted to diastereoisomers. Diastereoisomers usually differ in their physico-chemical properties, allowing their separation. In this case (**72**) and (**73**) were separated by column chromatography. This is not always possible and sometimes separation is achieved by first crystallising one diastereoisomer formed with, say, (–)-camphanic chloride, then recovering the parent alcohol enriched in the second isomer from mother liquors and recrystallising esters formed with (+)-camphanic chloride.

D- (**74b**) and L-2,4,5-tri-*O*-benzyl-1-*O*-(*p*-methoxybenzyl)-*myo*-inositol (**74a**) were obtained by ester hydrolysis, but it was not known which enantiomer was which. This can be determined either by obtaining a crystal structure of the parent diastereoisomer, or, if suitable crystals are unavailable, by converting one of the compounds to a known chiral intermediate. In this case benzylation and acidic hydrolysis of (**74b**) provided 1,2,4,5,6-penta-*O*-benzyl-*myo*-inositol (**75**), identified as the 1L-(–)-enantiomer by comparing its specific rotation to literature values. Therefore, the absolute configurations of the resolved enantiomers could be deduced.

Acidic hydrolysis of the enantiomeric diols provided triols (**65a**) and (**65b**). Many phosphorylation methods are available (reviewed[11]), but the one used in this example employed a P(III) reagent. Bis(benzyloxy)(diisopropylamino)phosphine was reacted with 1*H*-tetrazole to provide a tetrazolide intermediate. One of the chiral triols was then added to rapidly provide a trisphosphite. This was oxidised to the fully protected trisphosphate (**76a** or **b**) with *t*-butyl hydroperoxide.

The required enantiomeric trisphosphates (**55a**) and (**55b**) were then obtained by use of sodium in liquid ammonia, which cleaved the three benzyl ethers and six benzyl esters in a single step. The trisphosphates were purified from the basic resultant solution by ion-exchange chromatography.

Thus, from *myo*-inositol, thirteen steps were necessary to provide a chiral trisphosphate, and a further two were required to establish the absolute stereochemistry of the resolved intermediate.

1.5 Aims of the Project

Results of structure-activity studies at the Ins(1,4,5)P_3 receptor outlined in this chapter had indicated that important structural features for agonism were a D-*threo* bisphosphate arrangement as an absolute requirement, while a third phosphate and a hydroxyl group increased potency. Synthesis of chiral analogues from *myo*-inositol requires resolution of intermediates, which can be a difficult, tedious and expensive process.

Positions 4, 3 and 2 of D-glucose possess the same relative stereochemistry as positions 4, 5 and 6 respectively (and positions 4, 5 and 3 respectively) of D-*myo*-inositol derivatives. Therefore, use of D-glucose as starting material ought to eliminate the necessity of resolution. It was intended to examine regioselective protection of carbohydrate precursors and to utilise various carbohydrate rearrangement methodology to furnish selectively protected, chiral cyclohexane or cyclopentane derivatives, which could be further elaborated to target tris- or tetrakisphosphates. A fundamental objective was to ascertain whether compounds quite diverse from Ins(1,4,5)P_3, but retaining the pharmacophore, would exhibit agonism.

The first target was D-2-deoxy-*myo*-inositol-1,3,4,5-tetrakisphosphate, an analogue of the naturally occurring Ins(1,3,4,5)P_4, about which there was some controversy in the literature regarding its Ca^{2+}-mobilising ability and biological function (reviewed in chapter two). 2-Deoxy-Ins(1,3,4,5)P_4 could potentially be prepared by converting a selectively protected carbohydrate precursor to a 2-deoxyinososse using the Ferrier rearrangement (reviewed in chapter two).

A modification of the Ferrier rearrangement could be explored to attempt to prepare a versatile, selectively protected, chiral inositol intermediate suitable for the synthesis of several Ins(1,4,5)P_3 analogues.

The second target group of compounds were ring-contracted analogues of Ins(1,4,5)P_3. No cyclopentane-based compounds had previously been described, and these were potentially available using recently described ring-contraction techniques.

The discovery of the naturally occurring adenophostins, the most potent full agonists yet described (reviewed in chapter five), prompted investigations to prepare the third class of target compounds, based on glucose. The structure of the adenophostins was dissected to provide the most likely minimum active structure, and a route to this structure was designed, in addition to a versatile intermediate for synthesis of adenophostin analogues. Finally, a fluorescently labelled analogue of the above minimum structure was designed.

CHAPTER TWO

SYNTHESIS OF D-2-DEOXY-*myo*-INOSITOL-1,3,4,5-TETRAKISPHOSPHATE

2.1 INTRODUCTION

2.1.1 Metabolism of Ins(1,3,4,5)P_4

As outlined in chapter one, Ins(1,4,5)P_3 is metabolised by two pathways: hydrolysis of the phosphate at position 5 by a 5-phosphatase giving Ins(1,4)P_2 (**12**); or phosphorylation at position 3 by a 3-kinase giving Ins(1,3,4,5)P_4 (**13**). It is with the latter compound that this chapter is concerned.

Ins(1,3,4,5)P_4 was discovered in 1985,[120] and the kinase was subsequently characterised.[121,122] The metabolism of Ins(1,3,4,5)P_4 has been studied and is summarised in fig. 2.1. The main route for the production of Ins(1,3,4,5)P_4 is phosphorylation of Ins(1,4,5)P_3 by the 3-kinase enzyme. Ins(1,3,4,5)P_4 has also been isolated *in vitro* as a product of Ins(1,3,4)P_3 5/6-kinase action on Ins(1,3,4)P_3,[123] but this route is not believed to be physiologically significant.[123] The main route of Ins(1,3,4,5)P_4 catabolism is dephosphorylation by 5-phosphatase giving Ins(1,3,4)P_3 (ref. 52); it is also dephosphorylated by a 3-phosphatase, regenerating Ins(1,4,5)P_3.[124–126] It has been noted[52] that Ins(1,3,4,5)P_4 is probably not the physiological substrate of 3-phosphatase as the enzyme is inhibited by physiological concentrations of Ins(1,3,4,5,6)P_5 and $InsP_6$.[124] However, the activity of the phosphatase may be significant in experimental situations which involve broken and permeabilised cells (*vide infra*).

In 1992, when this project commenced, there were two controversies surrounding Ins(1,3,4,5)P_4. First, whether it possessed any physiological rôle (and what that rôle might be) and second, whether it could directly mobilise intracellular Ca^{2+} *via* the Ins(1,4,5)P_3 receptor. These two problems will be considered in turn.

2.1.2 Potential Rôles of Ins(1,3,4,5)P_4 and Putative Ins(1,3,4,5)P_4 receptors

The first suggestion that Ins(1,3,4,5)P_4 might possess an important physiological function followed the observation that a combination of Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 mimicked the effect of fertilisation in *Xenopus* oocytes, whereas no mimicry occurred with either compound alone.[127]

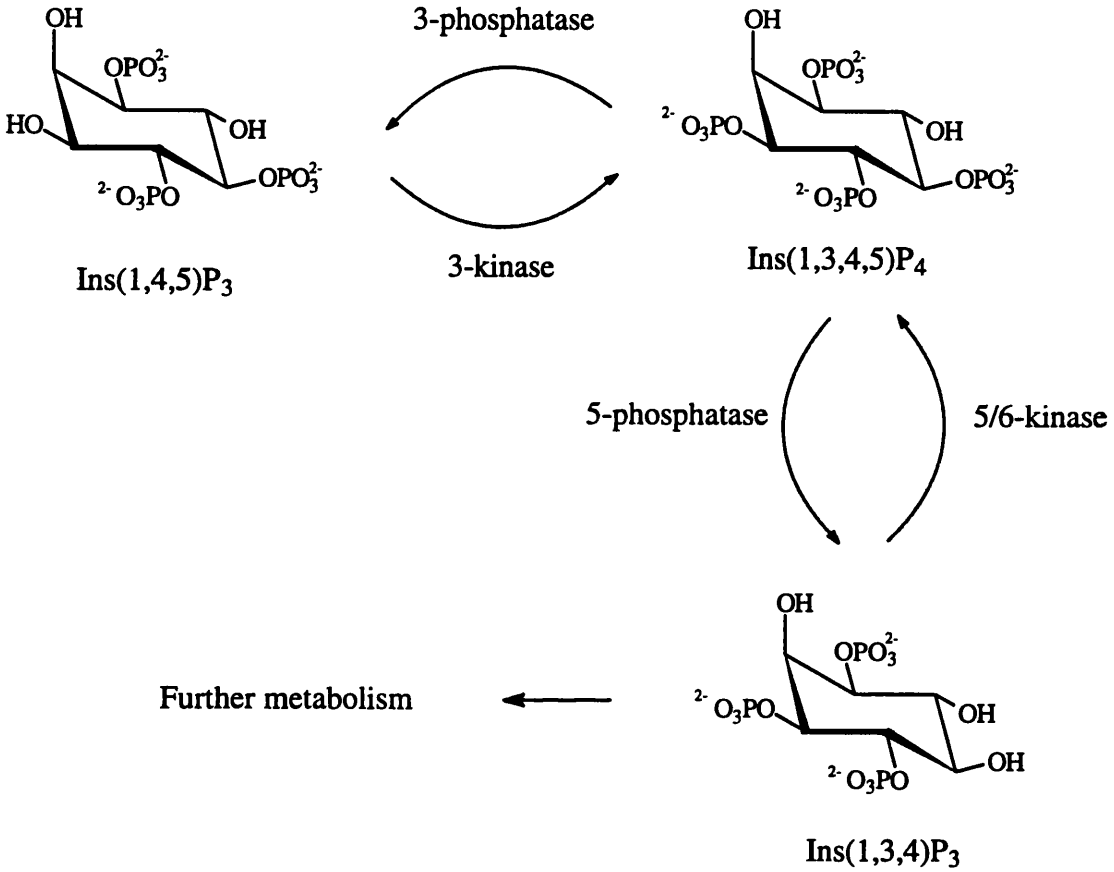


Fig. 2.1 Metabolism of Ins(1,3,4,5)P_4.

Subsequently, Hill *et al.*[128] presented evidence that Ins(1,3,4,5)P_4 induced Ca^{2+} sequestration into storage pools in rat liver cells. No other inositol phosphates tested were found to cause any sequestration.

It has also been suggested that Ins(1,3,4,5)P_4 is involved in Ca^{2+} homeostasis at the plasma membrane, helping to control entry of extracellular Ca^{2+} into the cell.[21,129]

Evidence against this hypothesis has been provided by Bird *et al.*,[130] who examined Ca^{2+} entry using Ins(2,4,5)P_3, an Ins(1,4,5)P_3 mimic which is not phosphorylated to the corresponding tetrakisphosphate. The trisphosphate alone was found to be necessary and sufficient for entry of Ca^{2+} across the plasma membrane.[130] In another study, the level of the 3-kinase enzyme present in NIH 3T3 fibroblasts and CCL39 cells was increased by transfection techniques, thereby generating high concentrations of Ins(1,3,4,5)P_4 at the expense of Ins(1,4,5)P_3.[131] There was no evidence that high Ins(1,3,4,5)P_4 levels promoted Ca^{2+} mobilisation, Ca^{2+} entry or Ca^{2+} sequestration, and it was concluded that Ins(1,4,5)P_3 was the major determinant in each case.

However, subsequently in support of the Ca^{2+}-entry hypothesis, Smith[132] found that Ins(1,3,4,5)P_4 could stimulate Ins(1,4,5)P_3-*insensitive* Ca^{2+} influx in mouse lacrimal cells. Additionally, an Ins(1,3,4,5)P_4-sensitive Ca^{2+}-permeable channel has been characterised from endothelial cells;[133] Ins(1,4,5)P_3 failed to induce an increase in this channel's activity.

One way to support the proposal of a physiological rôle for a ligand is to isolate its receptor. Intracellular sites that specifically bind Ins(1,3,4,5)P_4 in high affinity [and with high selectivity over Ins(1,4,5)P_3] have been noted in HL60 cells,[134] bovine adrenal cortex,[135] bovine parathyroid,[136] rat[137] and pig[138] cerebellum and human platelets.[139] Ins(1,3,4,5)P_4-binding proteins have been purified from pig[140] and rat[141,142] cerebellum and porcine platelets.[143] The last example has received particular recent interest: this protein has been tentatively proposed to be an Ins(1,3,4,5)P_4 receptor[143–145] and has demonstrated a high specificity for Ins(1,3,4,5)P_4 over all other inositol tetrakis-[144] and various other polyphosphates.[143] *In vitro* Ins(1,3,4,5)P_4-stimulated GAP activity against the oncogene *ras* has been demonstrated.[145] The Ins(1,3,4,5)P_4 binding region of this protein has been identified.[145] Evidence for the physiological significance of this protein together with the rôle of Ins(1,3,4,5)P_4 is awaited with interest.

Consistent with their proposed Ca^{2+}-entry function, Ins(1,3,4,5)P_4-binding proteins are located on the inner leaflet of the plasma membrane. However, it has yet to be unambiguously demonstrated that these proteins are not in fact receptors for

phosphatidylinositol 3,4,5-trisphosphate [PtdIns(3,4,5)P_3, **5**] another putative second messenger located in the plasma membrane,[8,15] of which Ins(1,3,4,5)P_4 represents the polar headgroup.

2.1.3 Ca^{2+}-mobilising capacity of Ins(1,3,4,5)P_4

Early studies investigating whether or not Ins(1,3,4,5)P_4 mobilised intracellular Ca^{2+} directly *via* the Ins(1,4,5)P_3 receptor produced conflicting results. Thus, it was reported that Ins(1,3,4,5)P_4 did not mobilise intracellular Ca^{2+} in Swiss 3T3 cells,[146] permeabilised Jurkat T-lymphocytes[147] or from aortic sarcoplasmic reticulum vesicles.[148] However, other studies reported direct Ca^{2+} release in cerebellar[149] and adrenal[150] microsomes, *Xenopus* oocytes[151] and permeabilised SH-SY5Y neuroblastoma cells.[95]

Based on the structure-activity relationships discussed in chapter one, Ins(1,3,4,5)P_4 should be an agonist at the Ins(1,4,5)P_3 receptor since it possesses the essential recognition elements, *i.e.* the D-*threo* bisphosphate arrangement at positions 4 and 5, a third phosphate at position 1 and a hydroxyl group at position 6. Indeed, given that the charged 3-*O*-methylenecarboxylate derivative (**40**) was found to mobilise intracellular Ca^{2+} in permeabilised SH-SY5Y neuroblastoma cells with an EC_{50} value of 3.50μM,[82] it would be surprising if the presence of a phosphate group at position 3 entirely abolished activity.

As the potency of Ins(1,3,4,5)P_4 was 10–100 fold lower than that of Ins(1,4,5)P_3 in the "positive" experiments, three potential reasons for false positive results could be identified:

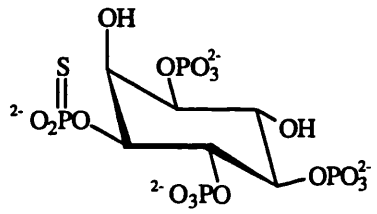
i) Were the Ins(1,3,4,5)P_4 preparations free from contamination by Ins(1,4,5)P_3?

ii) Was Ins(1,3,4,5)P_4 competing with Ins(1,4,5)P_3 for 5-phosphatase and thereby protecting Ins(1,4,5)P_3 from 5-phosphatase-catalysed deactivation?

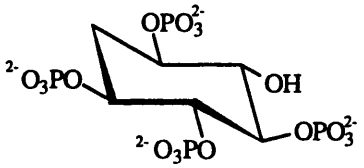
iii) Was endogenous 3-phosphatase converting Ins(1,3,4,5)P_4 back to Ins(1,4,5)P_3?

It was planned to clarify the situation by two approaches. First, to use pure, synthetic Ins(1,3,4,5)P_4 to eliminate any contamination problem. Second, to design and synthesise potential inhibitors of 3-phosphatase based on Ins(1,3,4,5)P_4. These would hopefully possess similar characteristics at the Ins(1,4,5)P_3 receptor, and would [in the absence of Ins(1,4,5)P_3] eliminate all three of the above problems.

Two potential 3-phosphatase inhibitors were designed. As replacement of the relevant phosphate group by a phosphorothioate had previously produced phosphatase inhibitors (*e.g.* Ins(1,4,5)P_3-5S inhibited 5-phosphatase[86]), Ins(1,3,4,5)P_4-3S (**77**) was an obvious target. Alternatively, deletion of hydroxyl groups adjacent to phosphomonoesters had also engendered inhibitory activity against phosphatases [*e.g.* 6-deoxy-Ins(1)P competitively inhibited inositol monophosphatase[152] and 6-deoxy-Ins(1,4,5)P_3 inhibited Ins(1,4,5)P_3 5-phosphatase[89]], so D-2-deoxy-Ins(1,3,4,5)P_4 (**78**) was another target.



Ins(1,3,4,5)P_4-3S (**77**)



D-2-deoxy-Ins(1,3,4,5)P_4 (**78**)

Compound (**78**) was also potentially useful to determine the importance of the hydroxyl at position 2 of Ins(1,3,4,5)P_4 with respect to binding to its putative receptor, similarly to the study of 2-deoxy-Ins(1,4,5)P_3 at the Ins(1,4,5)P_3 receptor.[67]

A route to (**78**) was explored and is described in this chapter.

A common route to chiral deoxyinositols is *via* Ferrier rearrangement of carbohydrate precursors. The Ferrier rearrangement is included in an extensive review of the conversion of carbohydrates to cyclopentane and cyclohexane derivatives.[153] As it

forms an important part of the work in two chapters of this thesis it will be briefly reviewed here, with an emphasis on relevance to this project, and to the field of inositol phosphate chemistry.

2.2 The Ferrier Rearrangement

2.2.1 Background

During the late 1970s, R. J. Ferrier was investigating potential routes to chiral cyclopentane derivatives from carbohydrates. He reasoned that a 6-deoxy-5-hexosulose derivative with a good leaving group at position 2 (*e.g.* **79**; fig. 2.2) might be induced to undergo a displacement reaction giving a cyclopentanone (*e.g.* **80**).

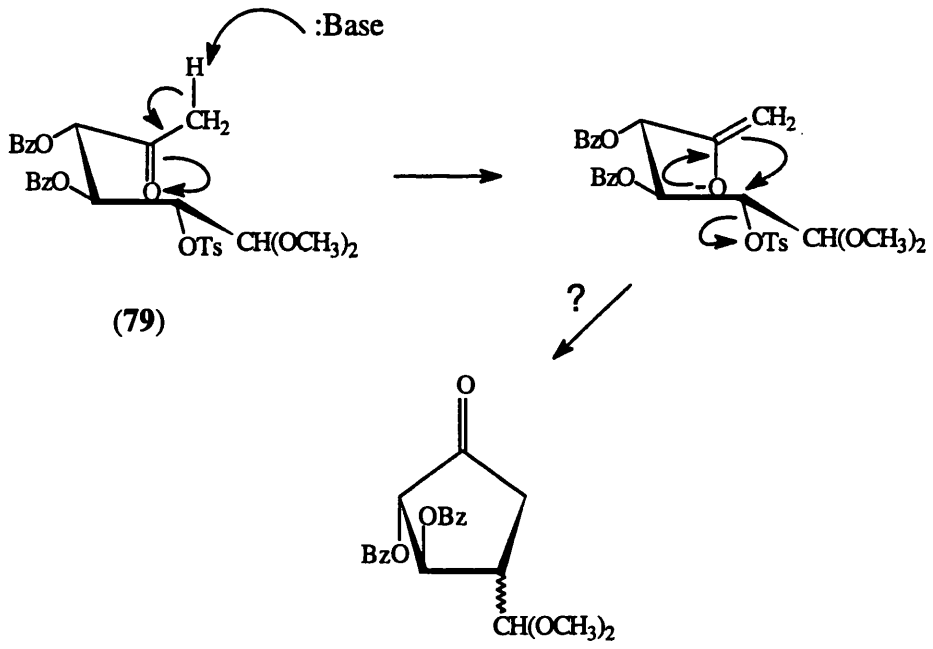
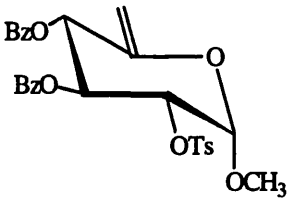
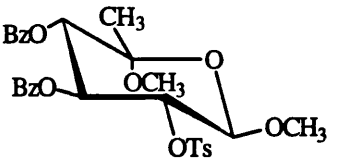


Fig. 2.2 Ferrier's proposed route to cyclopentanes from 5-hexosuloses.

Unexpectedly, treatment of enol ether (**81**) with methanolic HCl did not furnish (**79**), but (**82**).[154]

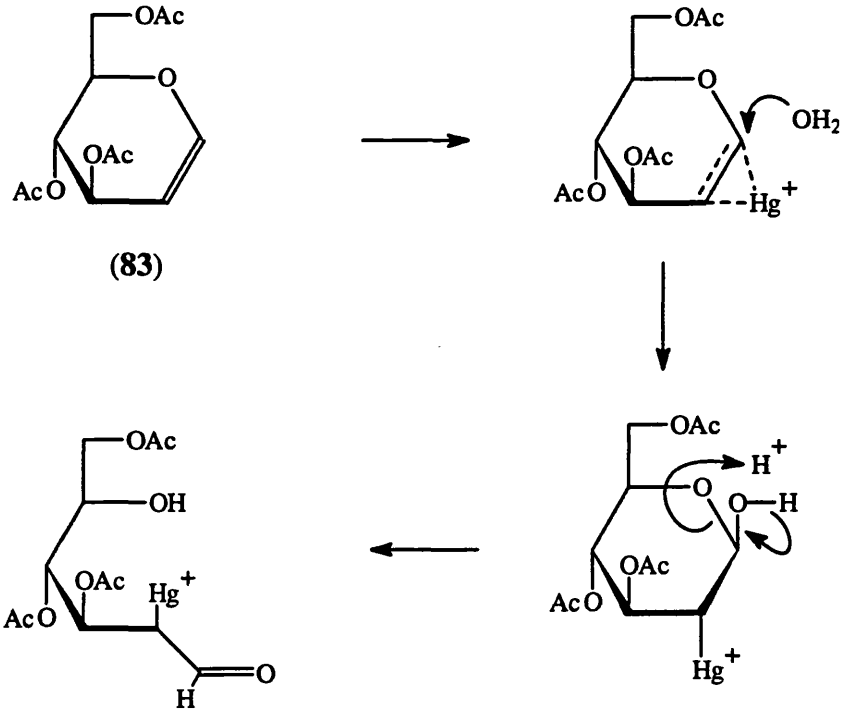


(81)



(82)

It was known that hydroxymercuration of carbohydrate derivatives such as D-glucal triacetate (**83**) leads to binding of the mercury (II) cation to the double bond, followed by ring opening (fig. 2.3).[155,156] Thus, Ferrier reasoned that hydroxymercuration of (**81**) would similarly generate a hemi-acetal at C-5 (fig. 2.4), with subsequent ring opening to give intermediate (**84**). In such a case C-6 would be expected to yield a potent carbanion, being stabilised both by a mercury ion and a carbonyl group. This anion might attack C-2 with elimination of tosylate as above to give the cyclopentanone (**80a**).



(83)

Fig. 2.3 Hydroxymercuration of D-glucal triacetate.

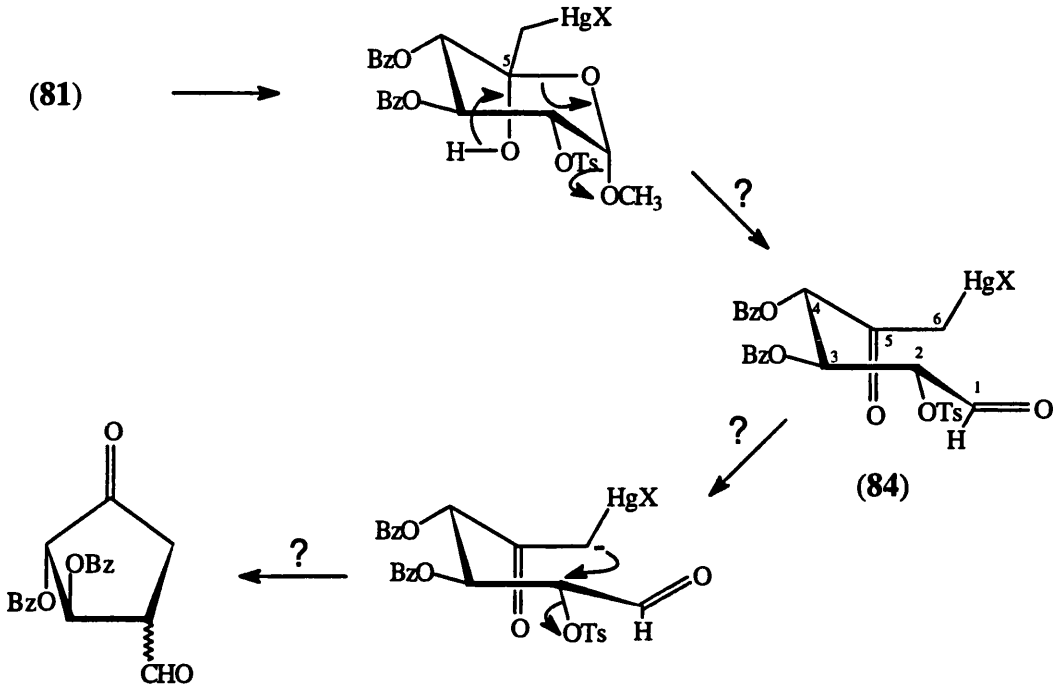
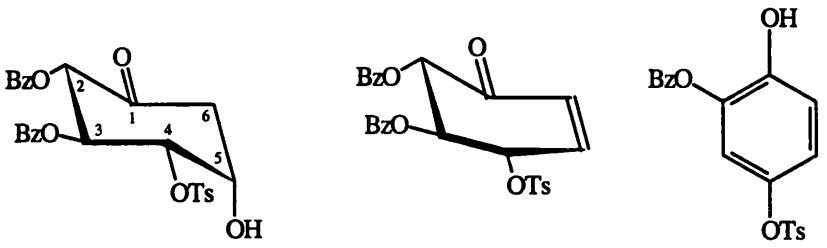


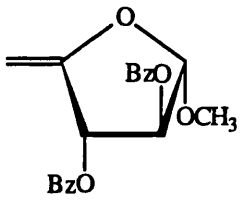
Fig. 2.4 Ferrier's potential route to cyclopentanes by hydroxymercuration of 6-deoxy-hex-5-enopyranosides.

Thus, (**81**) was heated under reflux in aqueous acetone (1:2) in the presence of an excess of mercury (II) chloride.[154] However, instead of the expected cyclopentanone, the substituted cyclohexanone (**85**) crystallised from the reaction mixture on cooling. The structure of (**85**) was unambiguously established by ^{1}H NMR spectroscopy, which indicated coupling constants consistent with a six-membered ring and not a five-membered ring; by ^{13}C NMR spectroscopy which indicated the presence of a carbonyl group; and by conversion *via* enone (**86**) to substituted benzene (**87**). This report also suggested stereospecificity of the rearrangement, thereby placing the C-5-OH *cis* to C-4-OTs as shown in (**85**).



(**85**) (**86**) (**87**)

Subsequent studies demonstrated that the initial hydroxymercuration occurred as predicted,[157,158] but the dicarbonyl intermediate (**84**) underwent an aldol-like closure, the nucleophilic centre at C-6 reacting with C-1 rather than C-2. Ferrier and Haines[159] have argued that the nucleophilic centre in the ring closure process can be assumed to possess enolate character as, when the C-6—Hg bond lengthens, the C-6—C-5 bond will shorten, transferring electron density to the carbonyl oxygen. In such a case, Ferrier's predicted closure [5-(enol *endo*)-*exo-tet*] is disfavoured according to Baldwin's rules,[160,161] whereas the observed closure [6-(enol *endo*)-*exo-trig*] is favoured.[161] It has been demonstrated that analogous cyclopentanones are *not* obtained from furanoside derivatives such as (**88**), which would have to undergo the disfavoured 5-(enol *endo*)-*exo-trig* ring closure and is therefore also consistent with Baldwin's rules.[161]



(**88**)

The observed Ferrier ring closure resembles the reaction catalysed by the NAD^+-dependent enzyme L-*myo*-inositol synthase, which converts glucose-6-phosphate (**89**) into L-*myo*-inositol-1-phosphate (**90**) *via* ring opening, a stereospecific intramolecular aldol ring closure and inosose reduction. This pathway has been reviewed[162] and is summarised in fig. 2.5. The ring closure also possesses similarities to the biosynthesis of shikimic acid (**91**) from D-glucose (summarised in fig. 2.6), in which a methylene enolate carbanion (**92**) is involved in an aldol-type ring closure to yield a cyclohexanone (**93**).[153]

2.2.2 Modifications of Original Conditions.

It soon became apparent that the rearrangement was not stereospecific as initially reported.[154,163] For example, an epimeric mixture of alcohols (**94**; major product) and (**95**) is obtained from (**96**),[164] and this is a general observation.

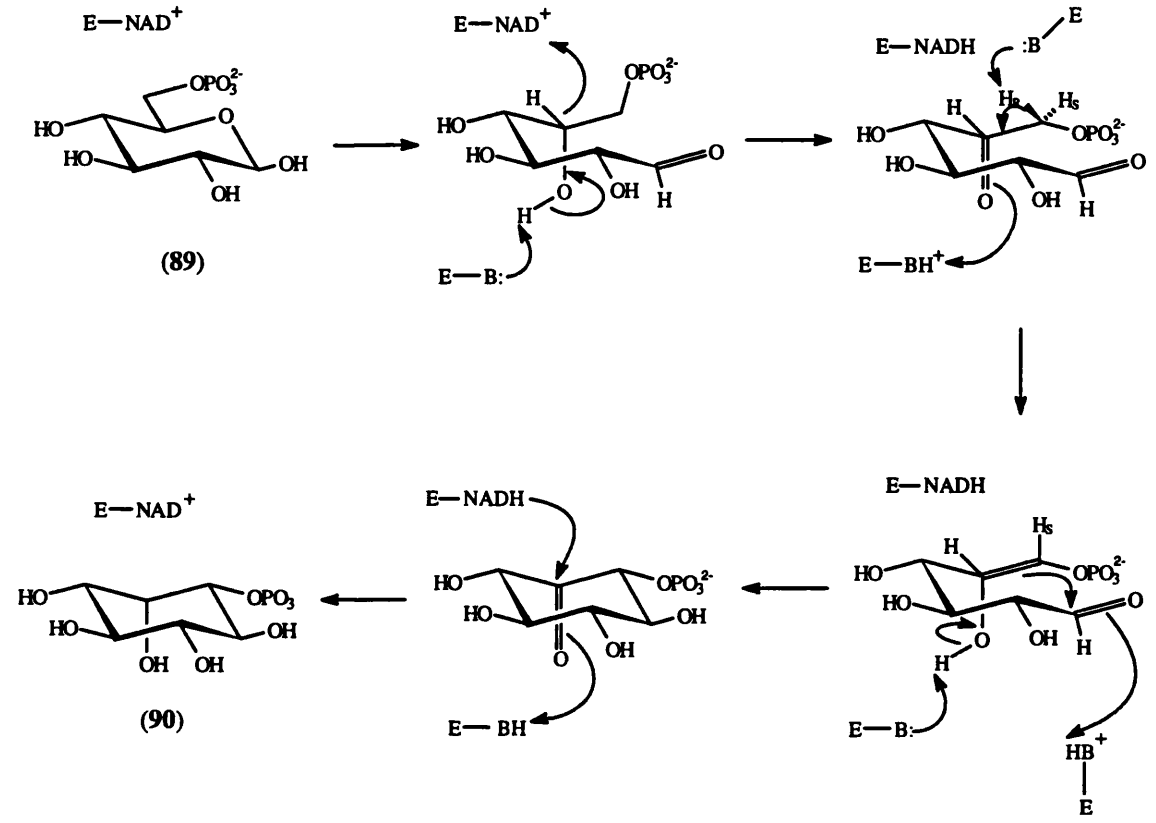


Fig. 2.5 Mechanism of conversion of D-glucose-6-phosphate to L-*myo*-inositol-1-phosphate by L-*myo*-inositol synthase.

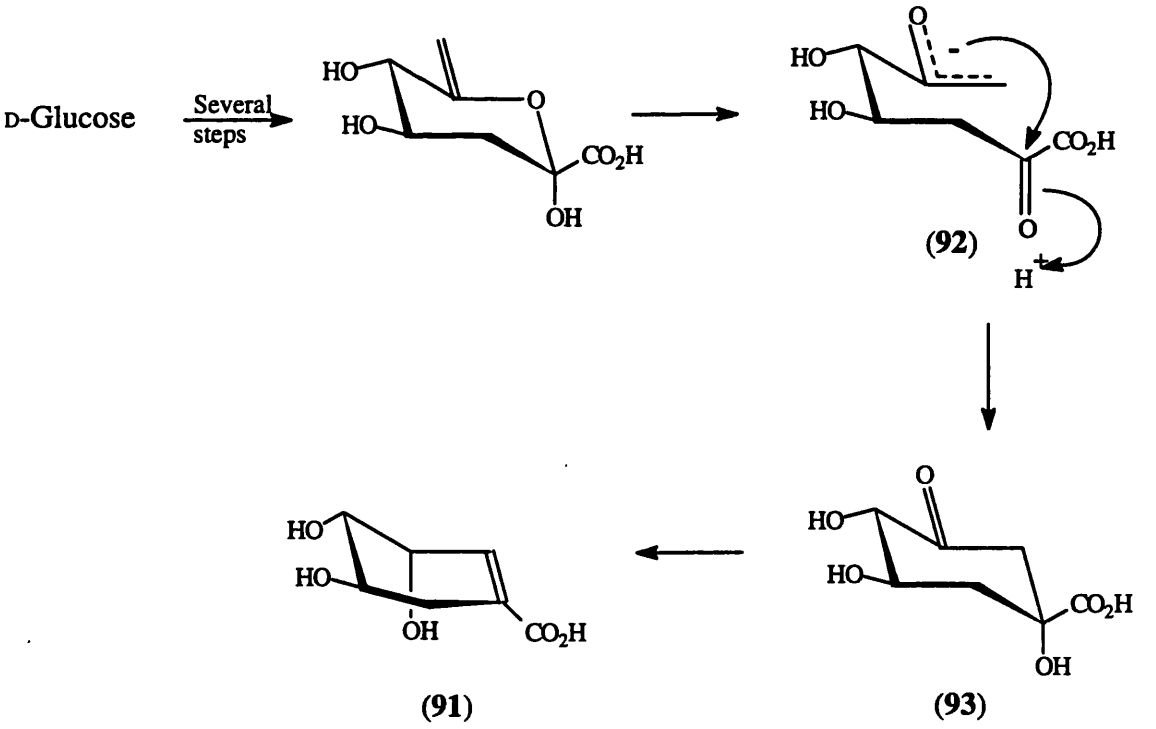
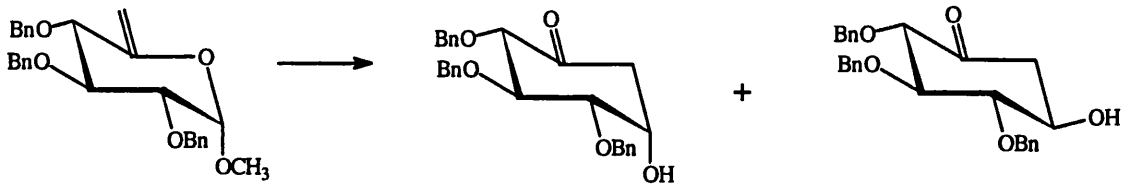
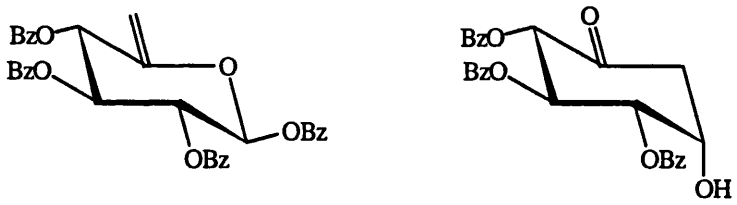


Fig. 2.6 Biosynthesis of shikimic acid.



(96) (94) (95)

In addition, some compounds gave erratic results with mercury (II) chloride,[158,165] or were difficult to isolate.[166] Chrétien and Chapleur[166] improved their yields by using mercury (II) trifluoroacetate at room temperature over a longer period. Concurrently, Ferrier's group established a greater efficiency (under refluxing conditions) with mercury (II) acetate.[158] It has been suggested[158] that the acetic acid produced in this case, being weaker than hydrochloric acid produced using mercury (II) chloride, is less likely both to reverse the original hydroxymercuration and to hydrolyse the mercurial adducts such as (**84**). Using this salt, (**97**) was prepared in 93% yield from (**98**), which was a considerable improvement on the mercury (II) chloride method.[158] However, although the hydroxymercuration step is rapid with mercury (II) acetates, the ring closure is sometimes more readily effected by addition of excess chloride ions.[158,167]

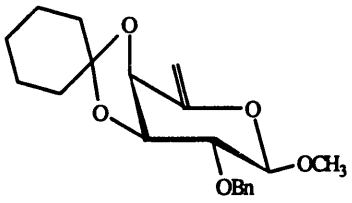


(98) (97)

Cleophax *et al.*[168] reported that methyl 2-*O*-benzyl-3,4-*O*-cyclohexylidene-6-deoxy-β-L-*arabino*-hex-5-enopyranoside (**99**) did not cyclise to (**100**) and (**101**) until addition of an excess of thiourea to the reaction mixture.

Lukacs and co-workers[165] demonstrated that the rearrangement also occurs smoothly under catalytic conditions. This group carried out the reaction at 60–80°C in 1,4-dioxane-5mM sulphuric acid in the presence of 2 mol% mercury (II) sulphate, conditions

which had previously been used as an alternative to refluxing aqueous acetone for hydroxymercuration of D-glucal.[156] It has since been demonstrated that, in the absence of acid, the reaction may be catalysed by various mercury (II) salts, of which the trifluoroacetate appears the most efficient.[169]



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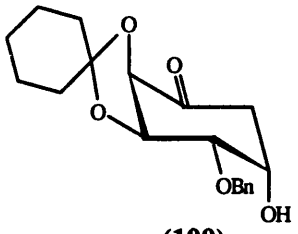
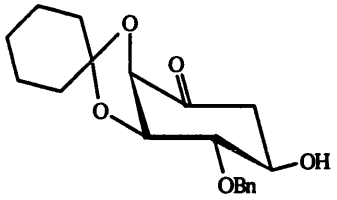
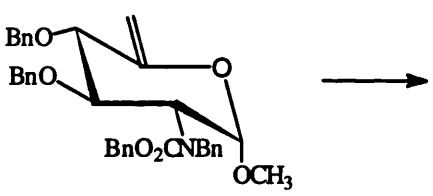


(100)

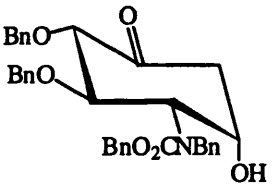


(101)

Adam[170] and Barton *et al.*[171] independently carried out both stoichiometric and (acidic) catalytic Ferrier rearrangements in the presence of palladium (II) salts. It is of particular interest that different ratios of epimeric products were obtained when some compounds were treated with palladium (II) chloride compared with mercury (II) sulphate, *e.g.* (**102**) and (**103**) from (**104**) table 2.1.[171]



(104)



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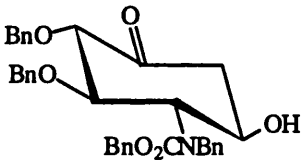


(103)

Conditions	(102)	(103)
a) Cat. $HgSO_4$, 5mM H_2SO_4, aq. dioxane, 2 h, 80°C, 78%	85	15
b) Cat. $PdCl_2$, 5mM H_2SO_4, aq. dioxane, 2 h, 80°C, 70%	60	40

Table 2.1 Ratios of major and minor cyclohexanone products of (**104**) obtained with mercury (II) sulphate and palladium (II) chloride.

2.2.3 Mechanism

Initial studies on the mechanism of the rearrangement were undertaken by Ferrier's group,[154,157-159,172,173] who established that isolable dicarbonylmercurial adducts such as (**84**) were formed. Machado *et al.*[174] recognised that the stereochemistry at position 5 of the cyclohexanone products was generally related to the conformation of the hex-5-enopyranoside precursors rather than their anomeric configuration. That is, carbohydrates adopting a $^4C_1$ conformation give predominantly the α-hydroxy epimer at position 5 (of the cyclohexanone product; see Appendix for nomenclature and numbering), while those having a $^1C_4$ conformation give predominantly the β-epimer (fig. 2.7).

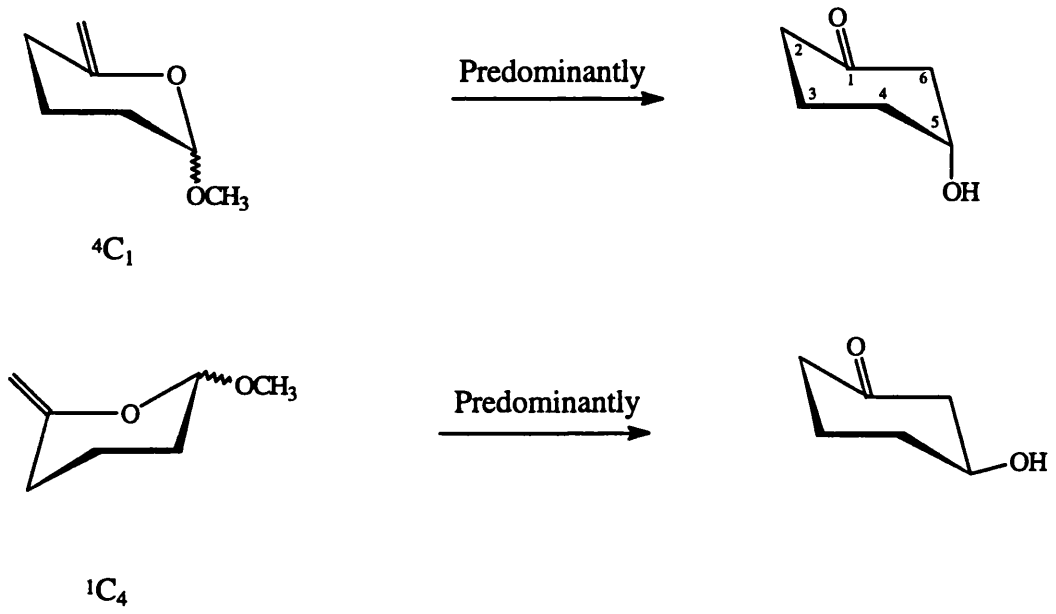


Fig. 2.7 Relationships between conformation of carbohydrate precursor and configuration at position 5 of cyclohexanone product.

László *et al.*[175] noted that the 5-hydroxyl and 3-substituent of cyclohexanone products tend to be *trans*. They suggested a mechanism in which the mercury atom of the dicarbonylmercurial intermediate co-ordinates to a lone pair-bearing substituent at position 3 (*e.g.* **105**, fig. 2.8). This directs the nucleophilic C-6 towards C-1 from the same side as C-3, giving a double boat conformation transition state (**106**).

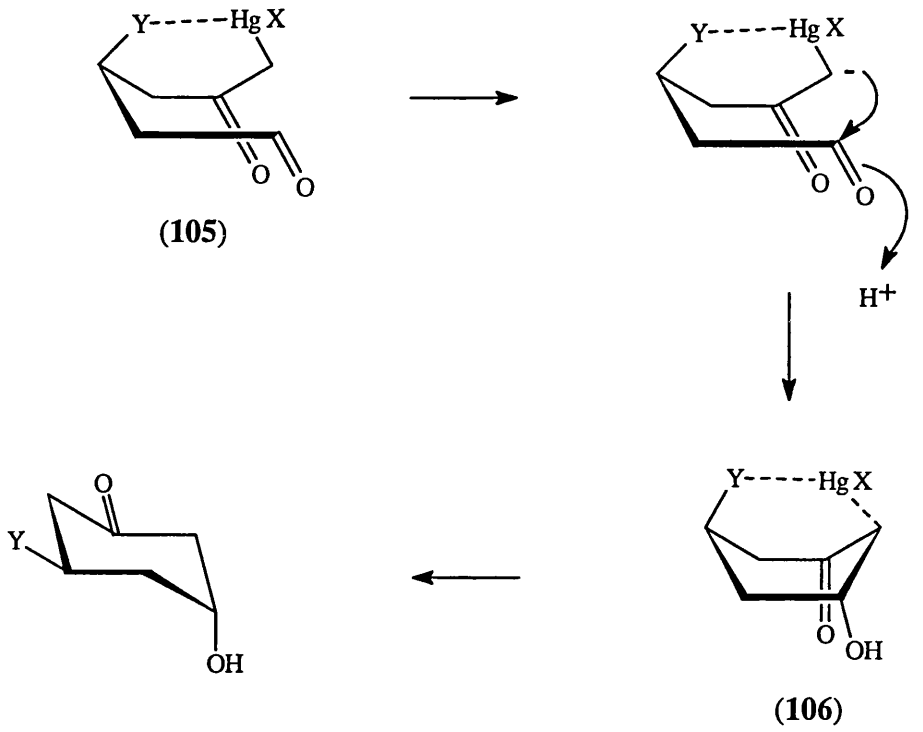
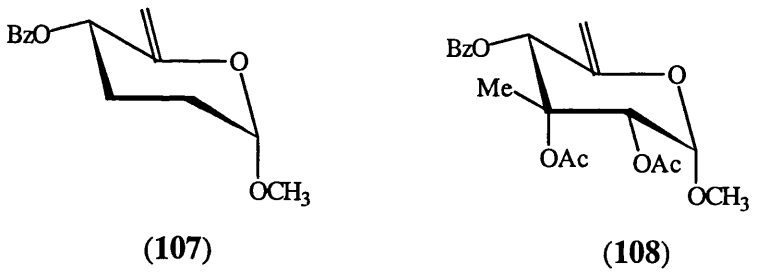


Fig. 2.8 Mechanism of the Ferrier rearrangement proposed by László *et al.*[175]



An example to support this hypothesis, methyl 4-*O*-benzoyl-2,3,6-trideoxy-α-D-*glycero*-hex-5-enopyranoside (**107**), lacks a 3-substituent and gives a 3:4 mixture of Ferrier products.[175] Additional support comes from compound (**108**), from which the corresponding 5β-hydroxycyclohexanone was the exclusive product.[176] In this molecule the mercury atom could co-ordinate to a lone pair on an oxygen atom of the 3-acetate, directing the β-product, but not to the methyl group which would produce the α-product.

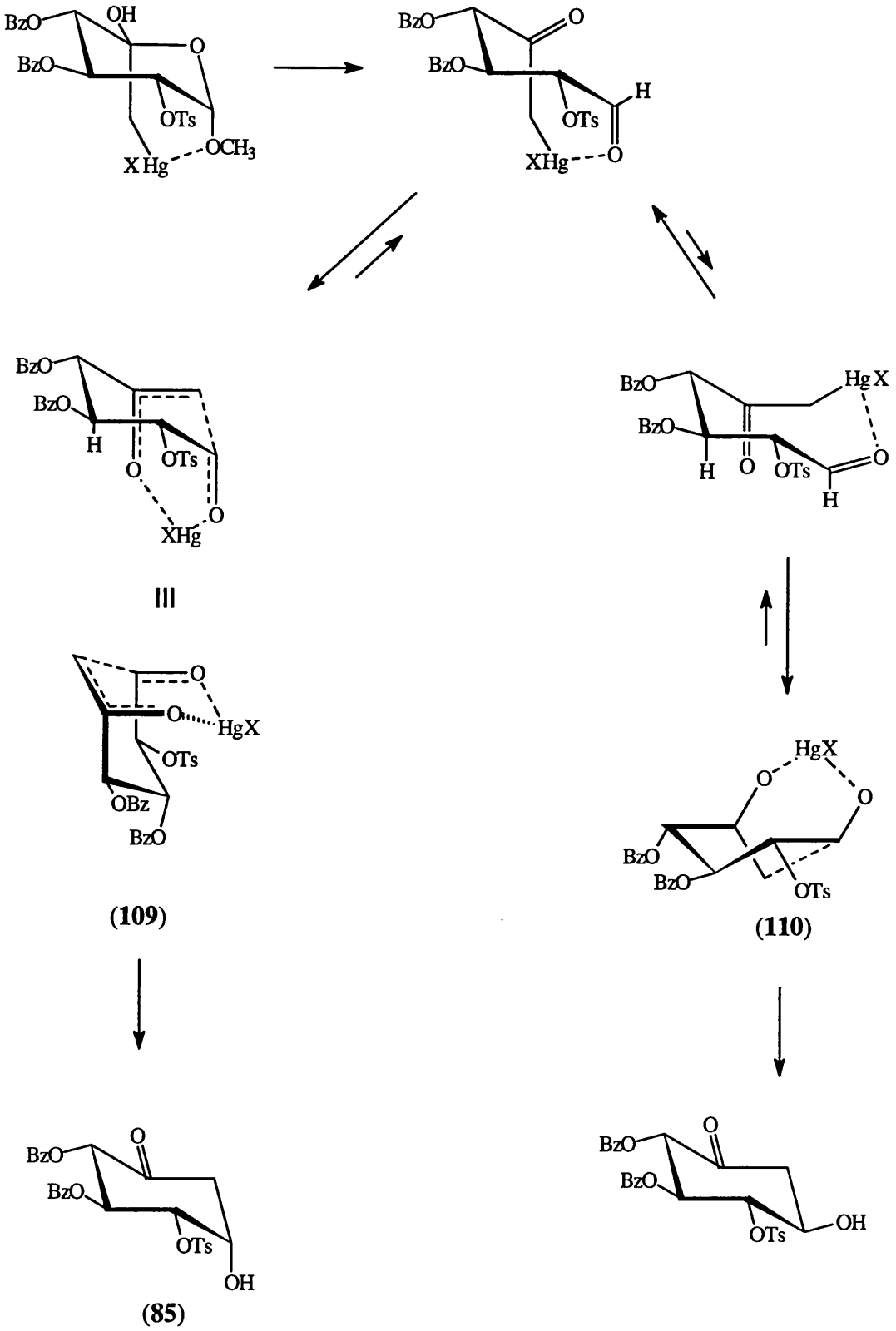


Fig. 2.9 Mechanism of the Ferrier rearrangement proposed by Machado *et al.*[177]

An alternative mechanism has been suggested by Machado *et al.*,[177] who propose one transition state involving two six-membered chairs formed by connection of carbonyl oxygen atoms to the mercury cation (**109**, fig. 2.9) which gives rise to the major product, and a disfavoured eight-membered twist conformation (**110**) which yields the minor product. They account for the *trans* C-3–C-5 relationship by proposing that the 1,3-diaxial interaction produced by a *cis* arrangement would destabilise intermediate (**109**), thus shifting the equilibrium towards the minor product.

A third possible mechanism has been proposed by Yamauchi *et al.*,[178] who prepared the selectively deuterated hex-5-enopyranoside (**111**) and exposed it to the original conditions using mercury (II) chloride. Loss of stereochemical integrity of the methylene group occurred in the overall reaction. On the basis of isolated intermediates, the stereochemistry was suggested to be lost partly during the hydroxymercuration step (although note that the isolated intermediates were *methoxymercurated*) and partly as a result of homolytic cleavage of the Hg—C-6 bond of the dicarbonyl adduct (**112**) (fig. 2.10; enolisation was excluded as deuterium was retained), an energetically feasible suggestion based on the model compound (1-chloromercurio)acetone. These workers suggest the radical (**113**) is the reactive species in the ring closure.

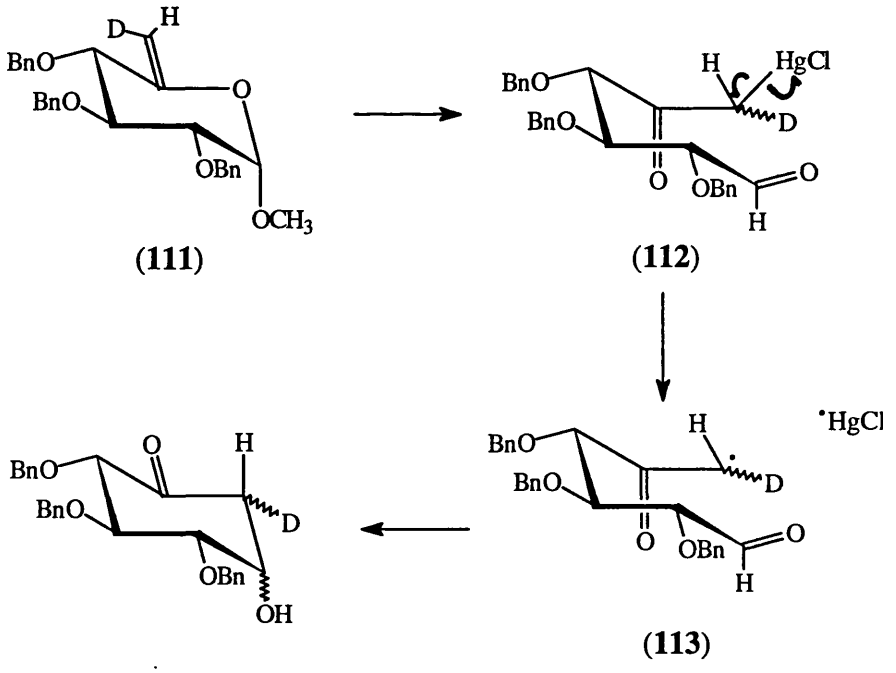


Fig. 2.10 Mechanism of Ferrier rearrangement proposed by Yamauchi *et al.*[178]

2.2.4 Applications to Inositol Phosphate Chemistry

The Ferrier rearrangement has found, and continues to find, many imaginative applications, notably allowing efficient access to modified aminocyclitol components of aminoglycoside antibiotics,[164,179–183] and to pseudo-sugars.[163,174,184–188] A thorough account of these areas is beyond the scope of this review, however, and only applications to the synthesis of inositol phosphates will be considered.

Sato *et al.*[189] unambiguously prepared L-1,2,4-tri-*O*-benzyl-*myo*-inositol (**114**; fig. 2.11), a phosphorylation precursor to Ins(1,4,5)P_3, from D-glucose. Methyl 2-*O*-benzyl-6-deoxy-3,4-di-*O*-(methoxymethyl)-α-D-*xylo*-hex-5-enopyranoside (**115**) was prepared from D-glucose in six steps. Ferrier rearrangement of (**115**) followed by β-elimination gave enone (**116**), which was reduced to the equatorial alcohol with complete stereoselectivity using $NaBH_4$-$CeCl_3$. After benzylation, treatment with osmium tetraoxide furnished *cis*-diol (**117**). Stannylene-mediated methoxymethylation exclusively gave (**118**), and benzylation followed by acidic hydrolysis provided (**114**).

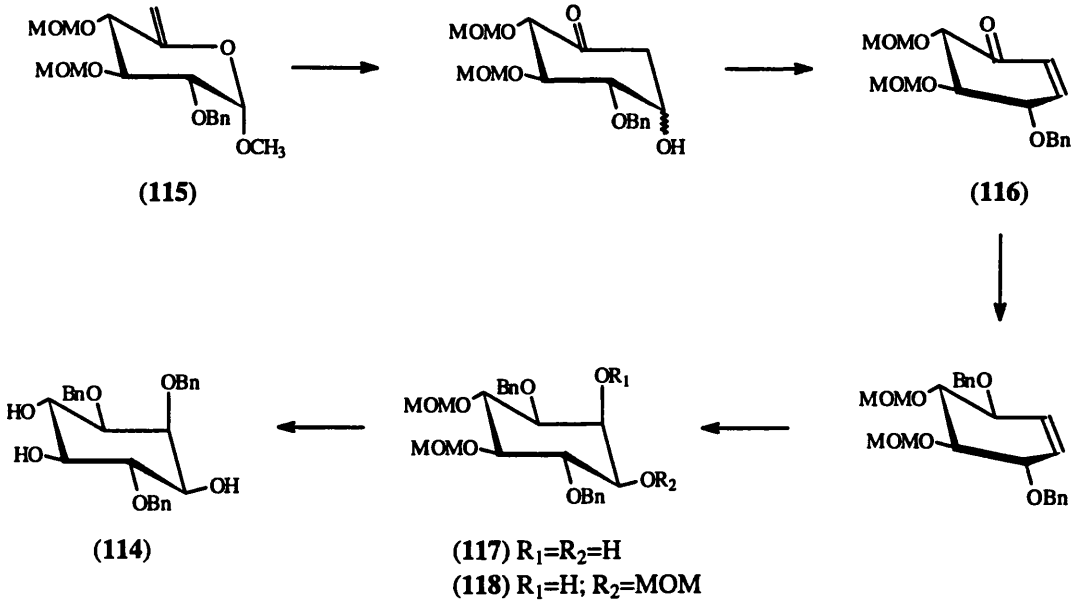


Fig. 2.11 Route to L-1,2,4-tri-*O*-benzyl-*myo*-inositol (**114**) from D-glucose by Sato *et al.*[189]

Several 6-deoxy-*myo*-inositol polyphosphates have been prepared from methyl β-D-galactopyranoside, by way of olefin (**99**).[168] A 2:1 mixture of the axial and equatorial products (**100**; fig. 2.12) and (**101**) respectively were obtained on modified Ferrier rearrangement (*vide ultra*). The equatorial product was reduced stereoselectively with lithium borohydride to give L-6-*O*-benzyl-1,2-*O*-cyclohexylidene-4-deoxy-*myo*-inositol (**119**), a versatile intermediate which was further elaborated to provide 6-deoxy-Ins(1)P (**120**), 6-deoxy-Ins(1,5)P_2 (**121**), 6-deoxy-Ins(1,4,5)P_3 (**47**), 6-deoxy-Ins(1,3,4,5)P_4 (**122**) and 6-deoxy-Ins(1,2-cyclic-4,5)P_3 (**123**). The biological properties of (**47**) were discussed in chapter one.

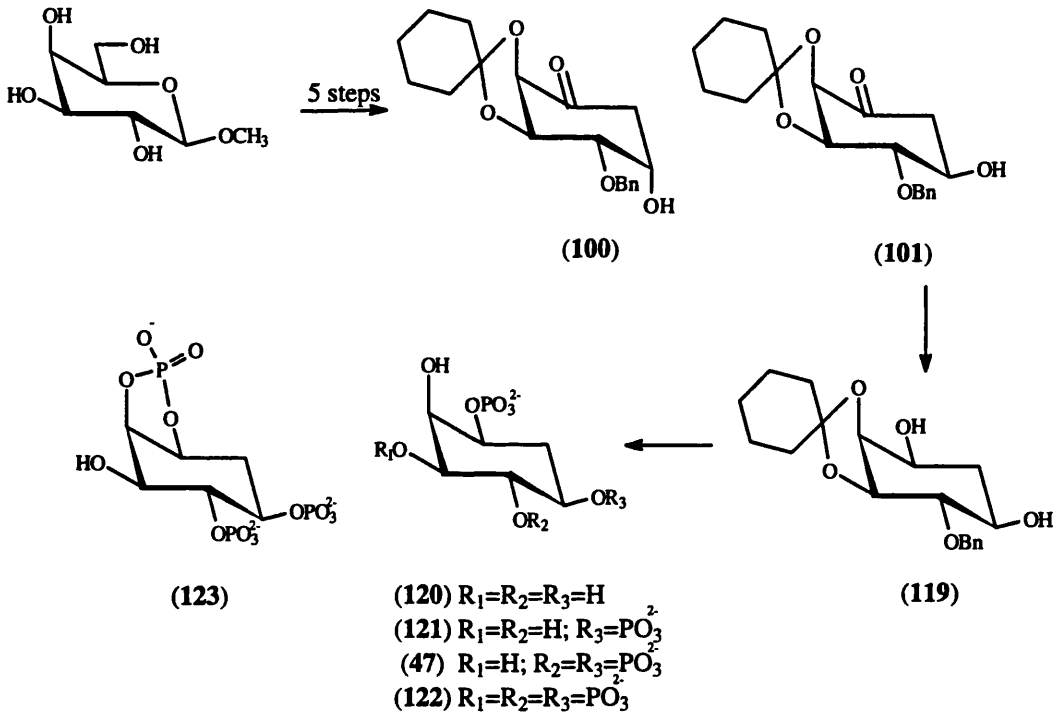


Fig. 2.12 Route to D-6-deoxy-*myo*-inositol polyphosphates from D-galactose by Cleophax *et al.*[168]

D-Glucose was used as starting material in a preparation of 3-deoxy-Ins(1,4,5)P_3 (**18**) and D-3-deoxy-*muco*-Ins(1,4,5)P_3 (**17**),[61] a synthesis noteworthy for incorporating allyl ethers in this mercury(II)-catalysed rearrangement. The anomeric mixture (**124**; fig. 2.13) gave (**125**) and (**126**) in a ratio of 6:1. Trisphosphate (**18**) was prepared by protecting the hydroxyl group of (**126**) as a tetrahydropyranyl ether. The ketone was

reduced to the axial alcohol, which was then benzylated followed by standard allyl/tetrahydropyranyl deprotection, then phosphorylation and complete deblocking. The *muco* analogue (**17**) was prepared from the major Ferrier product (**125**) similarly, except that the ketone was reduced exclusively to the axial alcohol in this case.

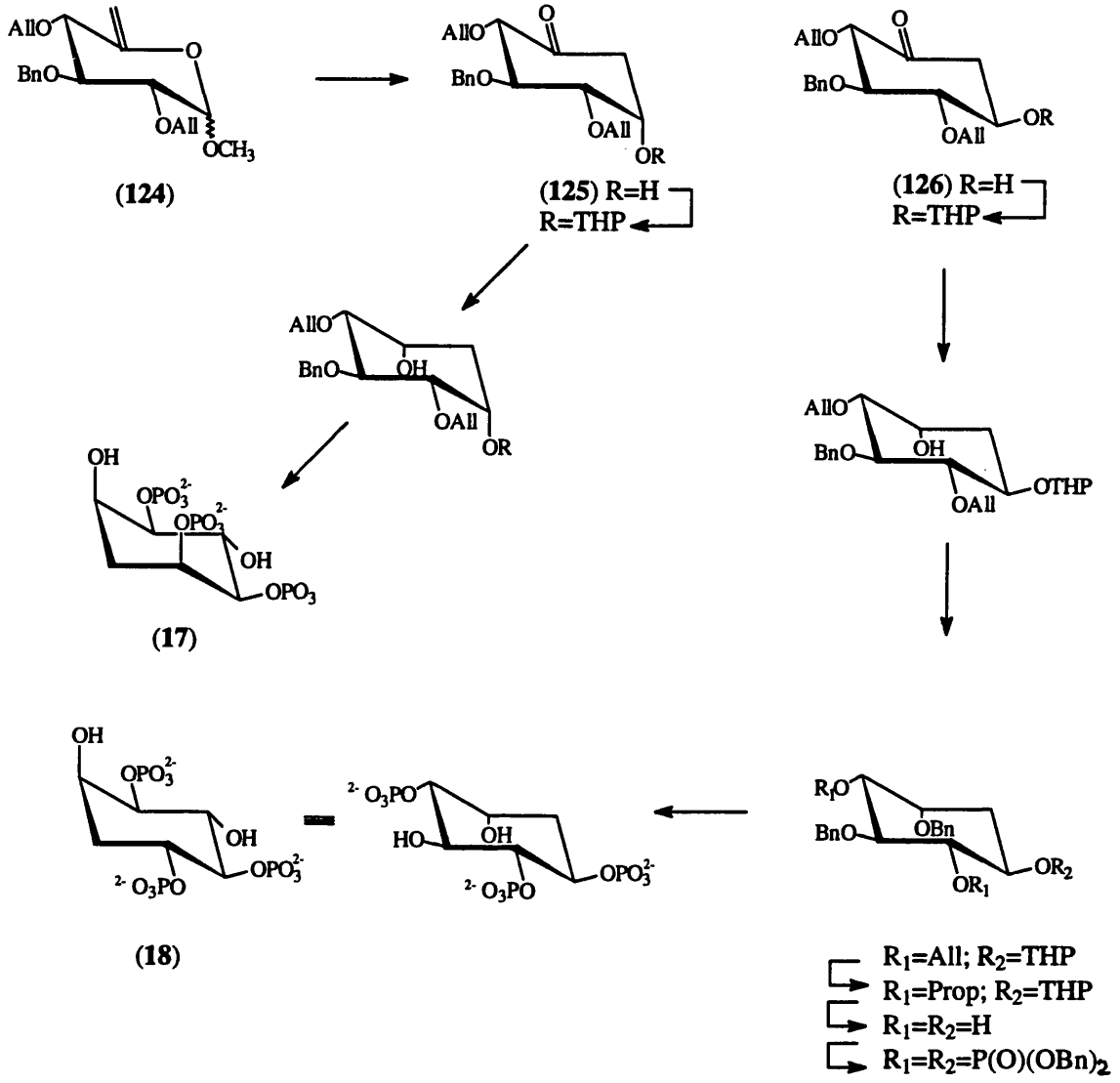


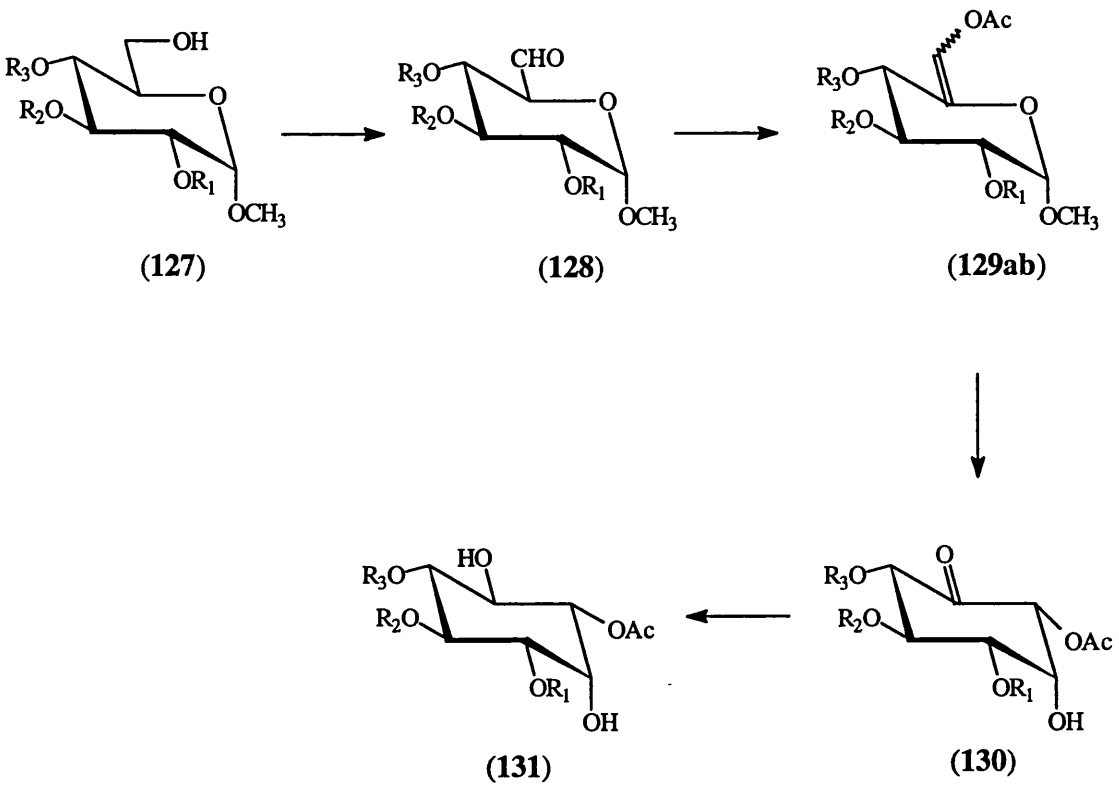
Fig. 2.13 Synthesis of 3-deoxy-*muco*- and *myo*-Ins(1,4,5)P_3 by Poirot *et al.*[61]

Bender and Budhu[167] devised an elegant biomimetic route to optically active *myo*-inositol derivatives. Various partially protected glucose intermediates (**127**; fig. 2.14; *e.g.* R_n=Bn) were oxidised to aldehydes (**128**), which were trapped as enolates by acetic anhydride to give a mixture of enol acetates (**129ab**). These underwent Ferrier rearrangement with high stereoselectivity for the D-*myo*-inos-6-oses (**130**), which could

be reduced stereospecifically with sodium triacetoxyborohydride to give *myo*-inositol derivatives (**131**).

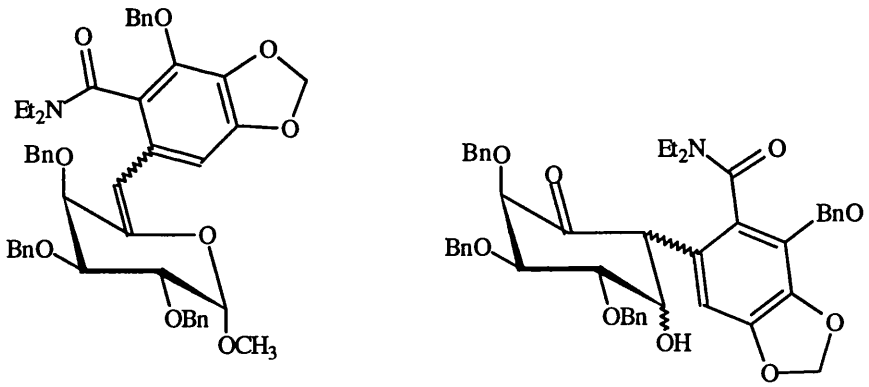
Of related interest is the recent report by Park and Danishefsky[190] in which the substituted enol ether (**132**) was subjected to classical Ferrier conditions to give (**133**). The axial or equatorial displacement of the aromatic group was not described.

Bender and Budhu's modification of the Ferrier rearrangement has been applied by Estevez and Prestwich[191] to prepare a chiral, P-1-tethered Ins(1,3,4,5)P_4 affinity label. The enol acetate (**129**; R_n=PMB) was converted to (**130**; R_n=PMB)and thence to (**131**; R_n=PMB). Protection of the hydroxyl groups as benzyloxymethyl ethers gave fully protected (**134**), which was saponified to (**135**). A protected (3-aminopropyl)phosphate was then introduced at position 1, and standard deprotection-phosphorylation chemistry supplied the P-1 aminopropyl tethered Ins(1,3,4,5)P_4 analogue (**136**). Coupling of (**136**) to a ^{125}I-labelled aminosalicylic acid derivative provided the target affinity label (**137**), which has been used to purify Ins(1,3,4,5)P_4 binding sites.[141,142] Intermediate (**135**) has also been used to prepare various 1-*O*-alkyl and 1-*O*-acyl analogues of PtdIns(3,4,5)P_3.[192]

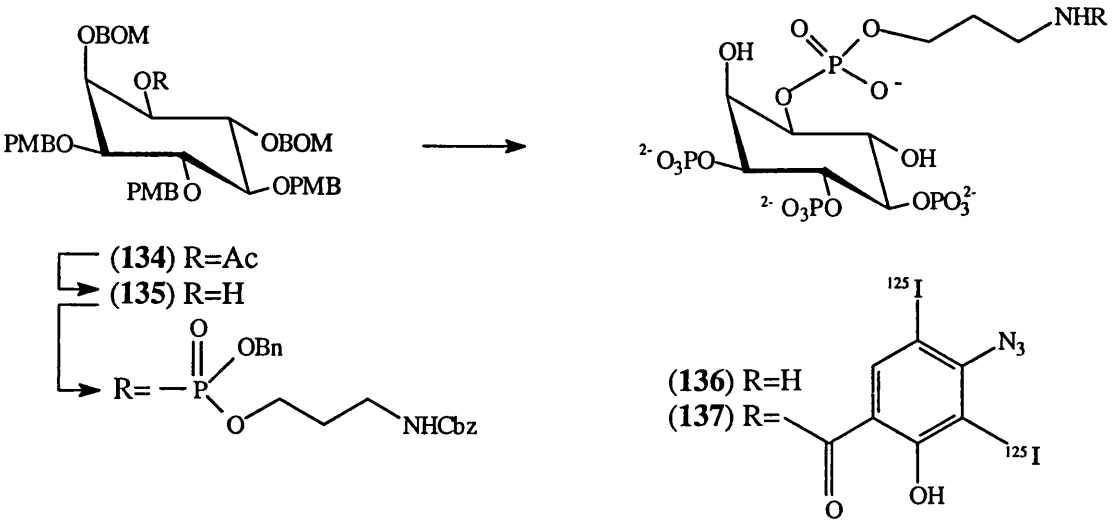


(127) (128) (129ab) (130) (131)

Fig. 2.14 Direct syntheses of *myo*-inositol derivatives by Ferrier rearrangement of enol acetates.



(132) (133)



Prestwich and co-workers[193] have used similar chemistry to prepare a P-5-tethered photoaffinity label of Ins(1,2,5,6)P_4 [Ins(1,2,6)P_3 inhibits inflammation and oedema in skin burn by acting as a neuropeptide Y antagonist][193] from methyl 2,3-di-*O*-benzyl-4-*O*-(*p*-methoxybenzyl)-α-D-glucopyranoside.

2.3 Retrosynthetic Analysis

It is clear from the above review that the Ferrier rearrangement may be useful to prepare enantiomerically pure 2-deoxy-Ins(1,3,4,5)P_4 from a suitably protected carbohydrate precursor.

D-2-Deoxy-Ins(1,3,4,5)P_4 (**78**; fig. 2.15) may be synthesised by phosphorylation of D-1-*O*-benzyl-3-deoxy-*scyllo*-inositol (**138**) followed by deprotection. All substituents in (**138**) are equatorial. This arrangement may be obtained by reduction of the ketone of a cyclohexanone Ferrier product in which all substituents are equatorial (*i.e.* **139**) to give the corresponding equatorial alcohol (**140**). Removal of protecting groups R, which must be orthogonal to benzyl ethers, would provide (**138**).

Cyclohexanones of type (**139**) are obtained as the minor products of the mercury(II) reaction on D-*xylo*-hex-5-enopyranosides (**141**), ultimately derived from D-glucose. To prepare (**78**), a methyl α-D-*xylo*-hex-5-enopyranoside derivative is required with a benzyl ether at position 2 and an alternative protecting group at positions 3 and 4.

Benzoate esters are easily introduced in high yield, orthogonal with respect to benzyl ethers, compatible with the conditions of the Ferrier rearrangement and often facilitate interpretation of ^{1}H NMR spectra. Benzoate esters were therefore chosen for this route.

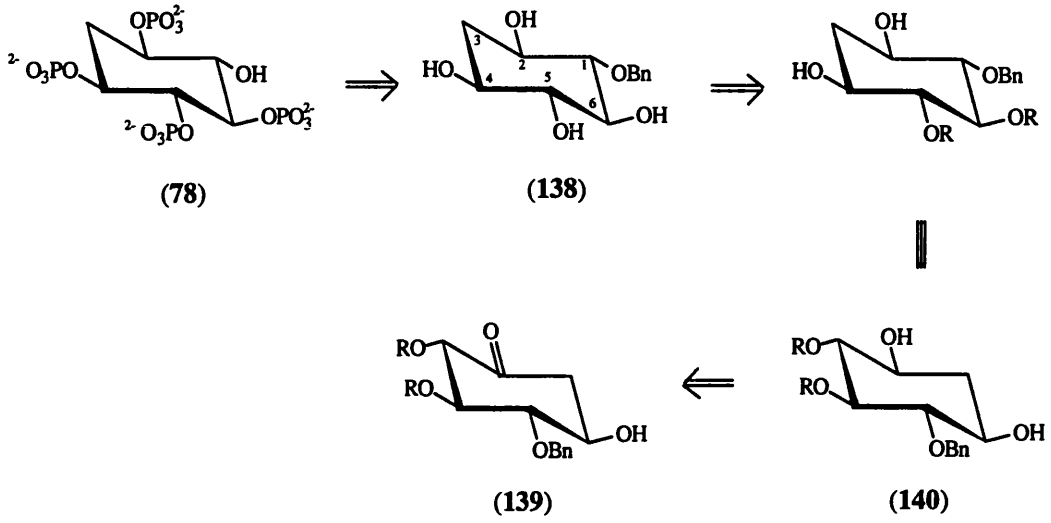
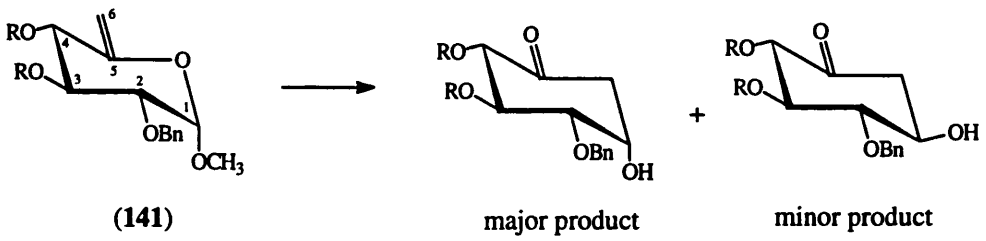


Fig. 2.15 Retrosynthetic analysis showing a potential route to (**78**) *via* Ferrier rearrangement.



The potential route envisaged to prepare D-(**78**) from methyl α-D-glucopyranoside (**142**) is outlined in fig. 2.16. This route involved benzylation of the 4,6-*O*-benzylidene derivative of (**142**), selection of the 2-*O*-benzyl product (**143**) and conversion *via* monobenzoate (**144**) to methyl 3,4-di-*O*-benzoyl-2-*O*-benzyl-6-bromo-6-deoxy-α-D-glucopyranoside (**145**) using standard carbohydrate chemistry. Elimination of HBr from (**145**) ought to provide the olefin (**146**), which on Ferrier rearrangement should give a mixture of C-5 epimeric cyclohexanones, (**147**) and (**148**). Inversion of stereochemistry at position 5 of the expected major product (**148**) and acylation would provide an intermediate (**149**). The stereochemistry at positions 2, 3, 4 and 5 of (**149**) is identical to

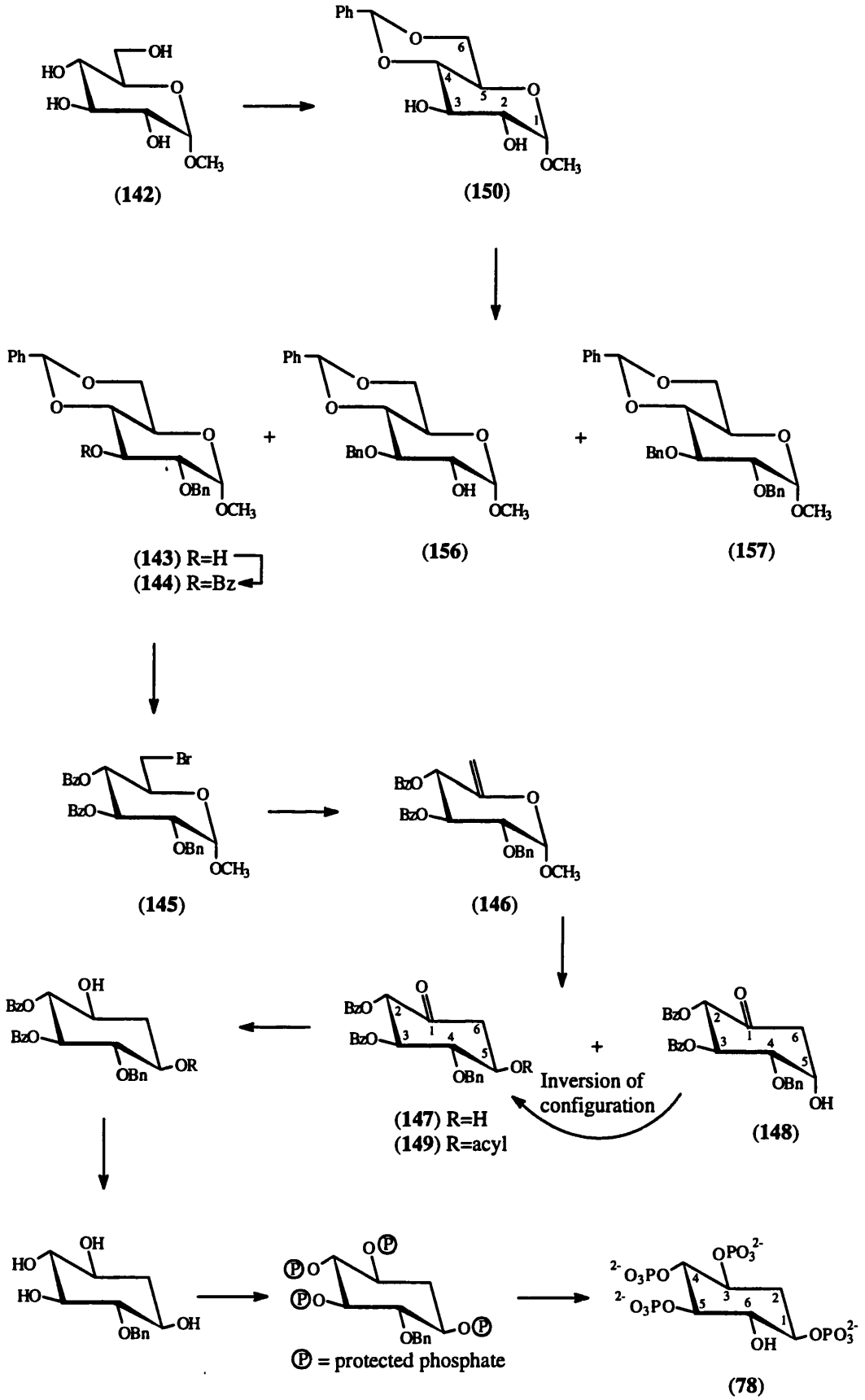


Fig. 2.16 Proposed route to D-2-deoxy-Ins(1,3,4,5)P_4.

that at positions 4, 5, 6 and 1 respectively of Ins(1,4,5)P_3. Additionally, the regiochemical protection would allow incorporation of phosphate groups into the appropriate positions. Intermediate (**149**) could therefore potentially furnish several useful polyphosphorylated compounds. In particular, stereoselective reduction of the ketone to the equatorial alcohol followed by ester hydrolysis, phosphorylation and deprotection should give (**78**).

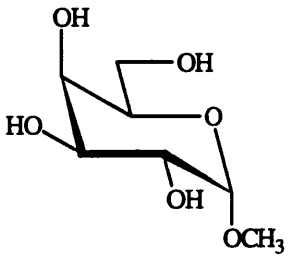
2.4 Discussion of Synthetic Work

2.4.1 An Improved Preparation of Methyl 4,6-*O*-benzylidene-α-D-glucopyranoside and Methyl 4,6-*O*-benzylidene-α-D-mannopyranoside

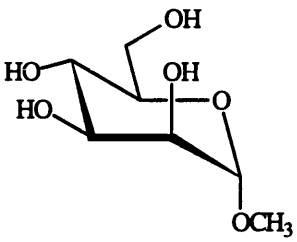
The benzylidene acetal is a commonly used protecting group for the 4,6-diol of hexopyranosides.[194] Many methods have been described for the preparation of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (**150**), including the reaction of methyl α-D-glucopyranoside (**142**) with zinc chloride-benzaldehyde[195,196] (and note an important modification[197]); with benzaldehyde dimethyl acetal in DMF in the presence of *p*-toluenesulphonic acid (PTSA)[198-200] or pyridinium *p*-toluenesulphonate[200] or tetrafluoroboric acid;[201] with benzaldehyde dimethyl acetal in 10% methanolic H_2SO_4;[202] and with benzaldehyde diethyl acetal in DMF in the presence of HCl,[203] or in dioxane in the presence of a strong cation exchange resin,[204] or in chloroform in the presence of camphorsulphonic acid.[205] Although excellent yields are reported for most of these techniques, their suitability to large-scale preparation (*i.e.* 0.5 mole or more) is rarely discussed.

In a synthesis of methyl 2,3:4,6-di-*O*-benzylidene-α-D-mannopyranoside (**151**), Horton and Weckerle[206] modified the method of Evans,[198,199] and stirred a mixture of methyl α-D-mannopyranoside, benzaldehyde dimethyl acetal, PTSA and DMF at 70°C with continuous removal of the liberated methanol *via* an air condenser attached to a water pump. This author applied their method[206] to methyl α-D-glucopyranoside, using 1.05 equiv. of benzaldehyde dimethyl acetal on a 0.5mole scale. The reaction was conveniently followed, being complete when methanol ceased condensing on the air

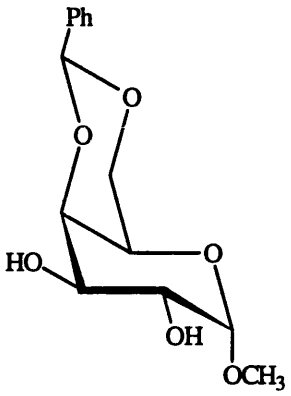
condenser. After evaporation of the solvent, the product was obtained in 90% yield as fine white needles by crystallisation from 2%$^{w}/_{v}$ aqueous sodium hydrogen carbonate solution. This improved preparation did not require dried solvent, or purified reagents.



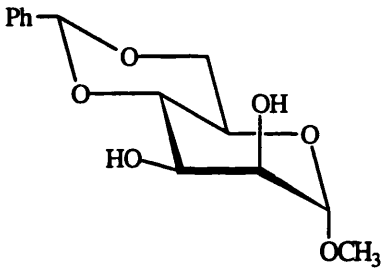
(152)



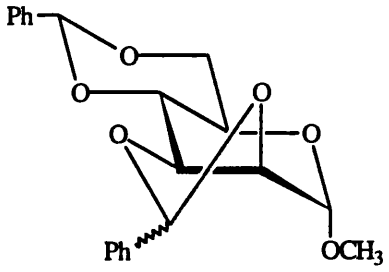
(153)



(154)



(155)



(151)

As this methodology was likely to be generally useful to carbohydrate chemists, the procedure was extended to the other two commonly used methyl hexopyranosides, methyl α-D-galactopyranoside (**152**) and methyl α-D-mannopyranoside (**153**). The galactoside gave a major product and a slightly less polar minor product (identified by Patroni *et al.*[200] as a diastereomeric mixture of the 3,4-*O*-benzylidene isomers) by TLC. The required 4,6-*O*-benzylidene derivative (**154**) did not crystallise from 2%$^{w}/_{v}$ aqueous sodium hydrogen carbonate solution, and could only be isolated pure after several crystallisations from ethanol. The yield was poorly reproducible, suggesting that other methods, notably benzaldehyde-zinc chloride,[207] may be superior.

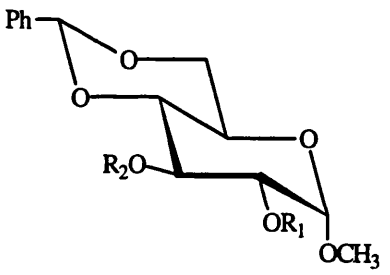
Methyl 4,6-*O*-benzylidene-α-D-mannopyranoside (**155**), however, was easily isolable by this method on a 0.5mole scale. When the reaction was completed, TLC indicated a major product corresponding to (**155**), a less polar minor product corresponding to the

dibenzylidene derivative (**151**)[206] and unreacted starting material. After evaporation of the solvent the residue was shaken with 2%$^{w}/_{v}$ aqueous sodium hydrogen carbonate solution and petrol, on the assumption that (**151**) would dissolve in the organic layer, while (**153**) would dissolve in the aqueous layer and (**155**) would precipitate. A precipitate was obtained, but contained a mixture of (**151**) and (**155**); pure (**155**) was obtained as fluffy white crystals by recrystallisation from chloroform-toluene. The yield (48%) was comparable to a 50mmol scale preparation by an adaptation of Evans' method,[208] and represents an improvement on a previous large-scale preparation using benzaldehyde-formic acid.[209]

2.4.2 Benzylation of Methyl 4,6-*O*-benzylidene-α-D-glucopyranoside and Related Reactions

With (**150**) in hand, attention turned to prepare the 2-*O*-benzyl derivative (**143**). Treatment of a solution of (**150**) in DMF with 1 equiv. of sodium hydride and 1.05 equiv. of benzyl bromide at room temperature gave 39% of (**143**), together with its 3-substituted isomer (**156**) (8%) and a relatively large proportion of the disubstituted derivative (**157**) (22%). As the yield of (**143**) was poor, a better benzylation method was sought. The monobenzyl ethers (**143**) and (**156**) have been prepared with little or no disubstitution using 1.1 equivalents of sodium hydride in neat benzyl chloride,[210] *via* phase transfer catalysis,[211] copper and mercuric chelates,[212] stannyl ethers[213] and stannylene acetals.[213] Of these, the last gave the highest reported yield of (**143**)* and was selected for further investigation. The selective manipulation of hydroxyl groups using organotin derivatives has been reviewed.[215] The dibutylstannylene intermediate (**158**; fig. 2.17; p.68) was formed by boiling a mixture of (**150**) and dibutyltin oxide in toluene for 2–3 h with azeotropic removal of water. The residue from this process was then heated with benzyl bromide in dry DMF to give (**143**) (46%) and (**156**) (19%) (table 2.2). No disubstitution occurred under these conditions. Slightly improved selectivity (and yield of **143**) was observed when the reaction was carried out in refluxing acetonitrile in the presence of quaternary ammonium salts, a procedure employed by Corrie[216] to *o*-nitrobenzylate (**158**).

* After completion of this work, Dasgupta and Garegg (ref. 214) reported a refinement of the stannyl ether method providing the best selectivity for, and yield of, (**143**) yet described.



(**159**) R_1=Bz; R_2=H
(**160**) R_1=Ts; R_2=H
(**161**) R_1=All; R_2=H
(**162**) R_1=H; R_2=All
(**163**) R_1=PMB; R_2=H
(**164**) R_1=H; R_2=PMB
(**166**) R_1=H; R_2=Bz

A claim by Boons *et al.*[217]** that benzylation of (**158**) (obtained from **150** and dibutyltin dimethoxide) in toluene at 50°C gave exclusively the 3-substituted product (**156**) could not be reproduced in this author's hands. Instead, an approximate ratio of 2.7:1, with (**156**) as the minor product, was obtained (table 2.2). In addition, treatment of the cooled solution of (**158**) with benzoyl chloride or tosyl chloride was found to give exclusively the 2-substituted product (**159** and **160** respectively) in both cases, in contrast to the above report.[217] The author's esterification results are consistent with those of a previous report,[218] which employed dioxane as solvent.

However, treating the stannylene (**158**) with benzyl bromide in dry DMF at room temperature in the presence of caesium fluoride,[219] did reverse benzylation selectivity. This procedure gave (**143**) and (**156**) in an approximate ratio of 1:2, a surprising

Alkylating reagent	Conditions	PRODUCT YIELD (%)	
		2-*O*-alkylation	3-*O*-alkylation
Benzyl bromide	b	46	19
	c	49	13
	d	41	15
	e	25	52
Allyl bromide	b	42	14
	e	19	40
p-Methoxybenzyl chloride	c	48	18
	e	17	25

Table 2.2 Yields of 2- and 3-*O*-alkylated methyl 4,6-*O*-benzylidene-α-D-glucopyranoside. (See experimental section for conditions. Yields are based upon isolated products).

** The *corrigendum* for ref. 217 was issued after communication of the results herein to the authors.

result as it is well established that, in the absence of steric effects, the 2-hydroxyl group is more reactive than the 3-hydroxyl in methyl α-D-glucopyranoside derivatives.[215,220,221]

Nagashima and Ohno[219] have suggested that caesium fluoride exerts its effect in two ways. First, that the caesium cation interacts with the bromine atom of benzyl bromide such that the electrophilicity of the benzyl methylene group is increased. Second, that the Sn—O bonds are activated by formation of a pentacoordinate complex involving two fluorine atoms. Applied to stannylene (**158**; fig. 2.17), it was reasoned that the bulky trigonal bipyramidal complex might be better accommodated at position 2 where steric interaction with the equatorial benzylidene acetal would be considerably reduced. Such an arrangement would yield the 3-*O*-benzyl product (**156**).

Such a mechanism would be expected to have little dependence on the alkyl halide used; the study was therefore extended to two other commonly used alkyl halides, allyl bromide (giving **161** and **162**) and *p*-methoxybenzyl chloride (giving **163** and **164**), and the caesium fluoride method reversed selectivity to some extent in both cases (table 2.2).

The argument above may be an oversimplification given the low solubility of caesium fluoride in DMF (0.3%$^{w}/_{w}$ at 80°C).[222]

Some generalisations observed by the author for the properties of 2- and 3-*O*-substituted derivatives of (**150**): 2-substituted derivatives tend to be less polar by TLC, possess lower melting points, exhibit sharper OH stretching bands in their IR spectra and have smaller $J_{H,OH}$ values than their 3-substituted isomers.

Attempts to isolate (**143**) from the purified reaction mixture by *crystallisation* met with limited success, since there was little consistency between successive experiments. However, after benzoylation of the purified mixture, the corresponding 3-benzoate ester (**144**) could be selectively obtained by fractional crystallisation from ethanol in 30–35% yield, a result reproducible on a 0.5mole scale. Compound (**144**) was identified from its

^{1}H NMR spectrum, which displayed a deshielded triplet at 5.85 ppm corresponding to the methine at position 3.

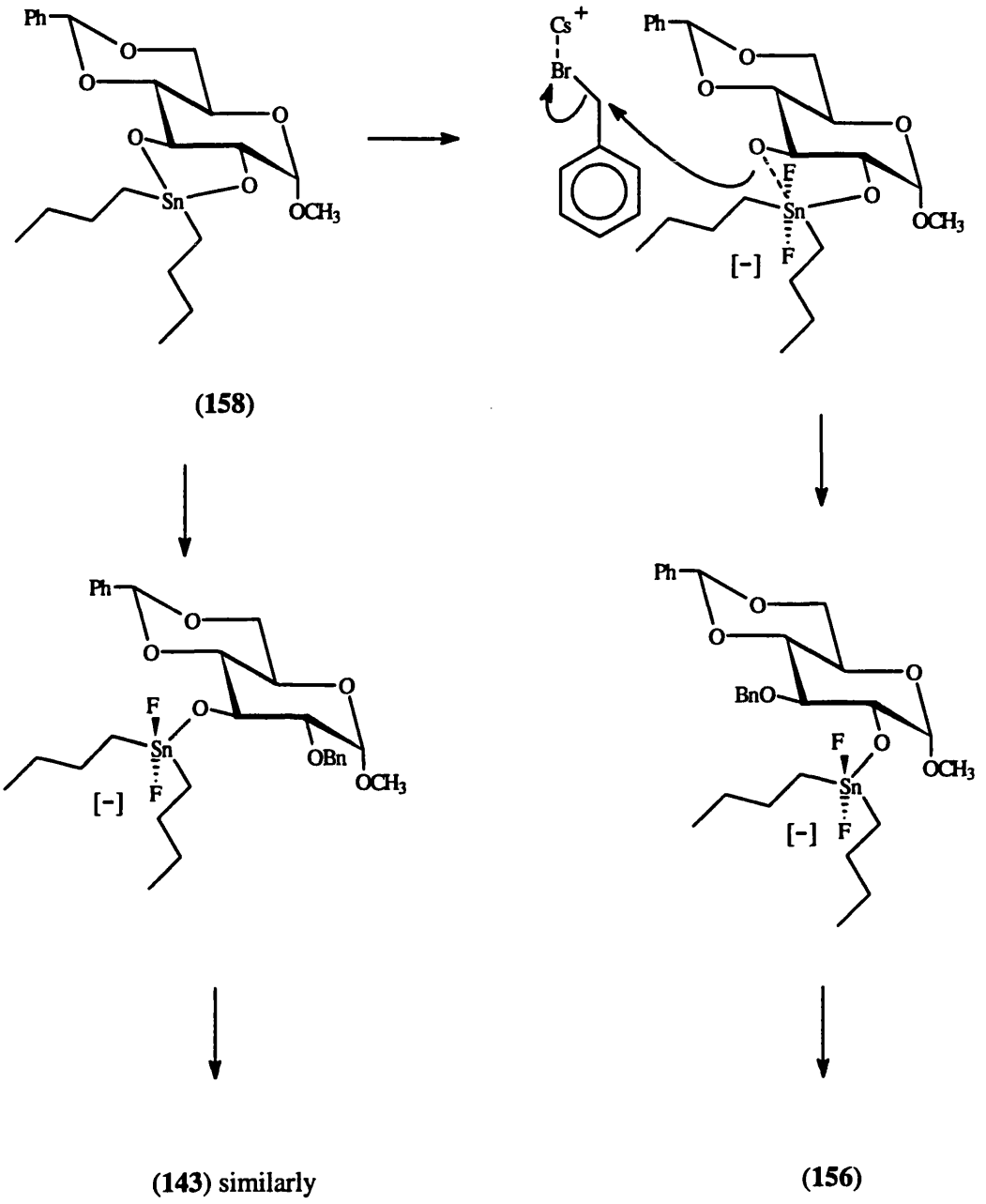
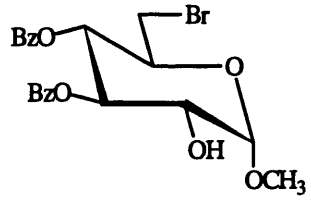


Fig. 2.17 Possible mechanism for benzylation of stannylene (**158**) using CsF.

2.4.3 Preparation of Methyl 3,4-di-*O*-benzoyl-2-*O*-benzyl-6-deoxy-α-D-*xylo*-hex-5-enopyranoside (146)

The conversion of 4,6-*O*-benzylidene hexopyranosides to 4-*O*-benzoyl-6-bromo-6-deoxy hexopyranosides by *N*-bromosuccinimide (NBS) in carbon tetrachloride containing excess barium carbonate (the "Hanessian–Hullar" reaction) was first described by Hanessian,[223] and a modified procedure subsequently by Hullar and co-workers.[224] This transformation has been widely used in carbohydrate chemistry and is applicable to large-scale procedures.[225]

A mixture of (**144**), NBS (1.2 equiv.) and barium carbonate (1.5 equiv.) was heated under reflux in dry carbon tetrachloride. The system changed in appearance from a white solid in a colourless solution to a pale yellow solid in a colourless solution followed by a white suspension in an intensely orange solution and then back to its original appearance within about 35 min, a characteristic sequence.[226] On purification, only 28% of the required bromo-dibenzoate (**145**) was obtained; the known methyl 3,4-di-*O*-benzoyl-6-bromo-6-deoxy-α-D-glucopyranoside[227] (**165**; 16%) and methyl 3-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranoside[228] (**166**; 10%), representing *O*-debenzylated product and starting material respectively, were also obtained, together with unreacted starting material (16%).



(**165**)

The structure of (**145**) was established chiefly by ^{1}H NMR spectroscopy and mass spectrometry. The presence of two benzoate esters was apparent by the four deshielded aromatic protons, corresponding to the *ortho* protons of the benzoyl group; the position of these esters at C-3 and C-4 of the pyranoside ring was confirmed by the two deshielded triplets (fig 2.18) at 5.28 and 5.94ppm; the presence of bromine in the

molecule was confirmed by the characteristic bromine isotope pattern in the mass spectrum.

The unwanted *O*-debenzylation side reaction was not entirely unexpected as the irradiation of mixtures of NBS, calcium carbonate and solutions of carbohydrates in aqueous carbon tetrachloride with a 375-W incandescent lamp has been used to selectively deprotect benzyl ethers.[229] In the hope that benzylidene cleavage occurred more rapidly than debenzylation, a fine suspension of NBS in carbon tetrachloride was added dropwise to a refluxing mixture of the other components. This failed to increase the yield of (**145**), as did more rigorous purification of reagents,[230] or using exactly one equivalent of NBS.

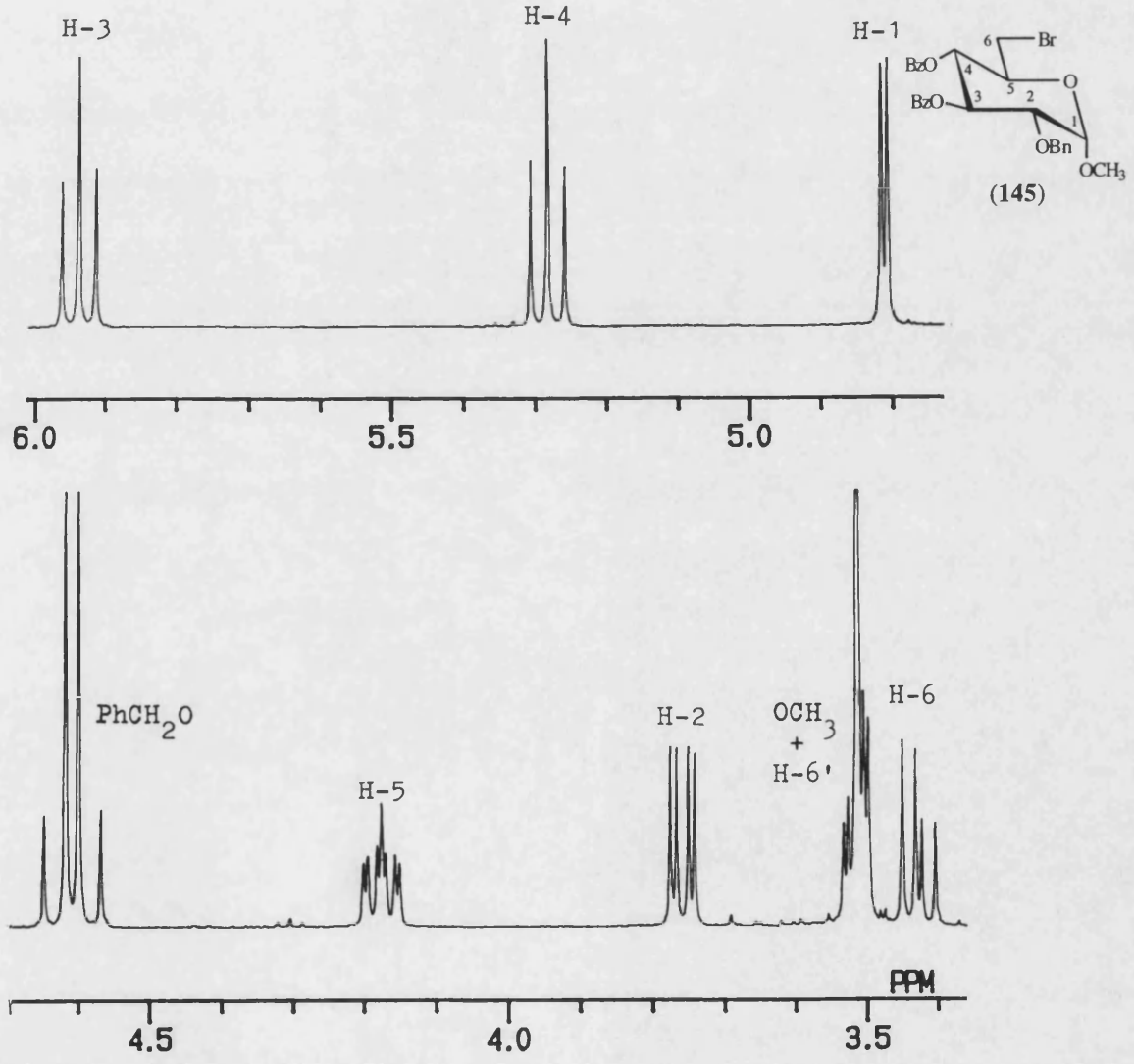
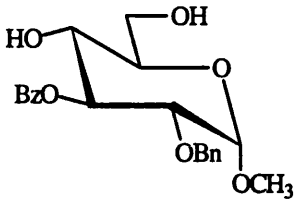


Fig. 2.18 Part of the 400MHz ^{1}H NMR spectrum of (**145**) in $CDCl_3$.

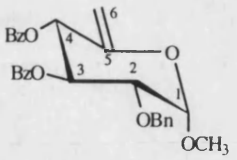
A more efficient route from (**144**) to (**145**) required acidic hydrolysis of the benzylidene acetal to give methyl 3-*O*-benzoyl-2-*O*-benzyl-α-D-glucopyranoside (**167**), followed by bromination with triphenylphosphine-carbon tetrabromide in THF[231] and conventional benzoylation.



(**167**)

Elimination of HBr from (**145**) to provide the Ferrier precursor (**146**) was initially attempted using silver (I) fluoride in dry pyridine.[232] However, for complete reaction at least six equivalents of the expensive silver salt were required, and a poor yield was obtained (31% after crystallisation). Interhalogen exchange with sodium iodide in acetone followed by treatment with DBU in refluxing toluene failed to effect complete conversion, but interhalogen exchange at 100°C in DMSO containing molecular sieves, followed by addition of DBU[233] worked efficiently, giving (**146**) in 63% yield after crystallisation. Olefin (**146**) crystallised readily from ethanol and was perfectly stable at room temperature in its crystalline form.

The structure of (**146**) was assigned as follows. The TLC chromatogram stained brown with potassium permanganate solution. The IR spectrum displayed the characteristic[163] hex-5-enopyranoside C=C enol ether stretching at 1670cm^{-1}. The ^{1}H NMR spectrum (fig. 2.19) displayed two one-proton triplets (*J* 2.0Hz for each proton) at 4.62ppm and 4.79ppm corresponding to the position 6 methylene, in which the geminal olefinic coupling was equal to four bond couplings to the position 4 methine; this methine presented as a doublet of triplets, with an axial-axial coupling to position 3, together with the long range coupling described above. In the ^{13}C NMR spectrum the methylene C-6 resonated at 97.34ppm and the quaternary C-5 at 150.30ppm.



(**146**)

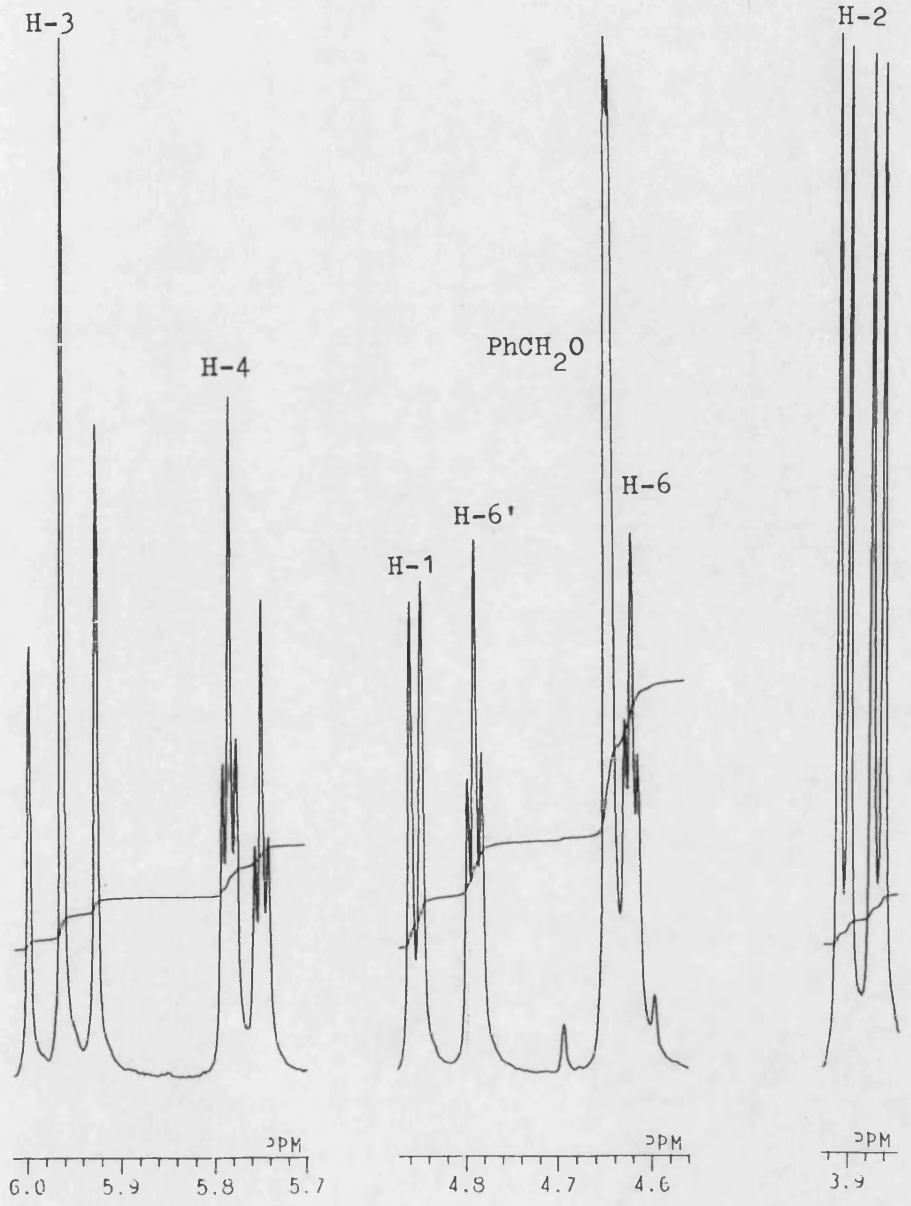


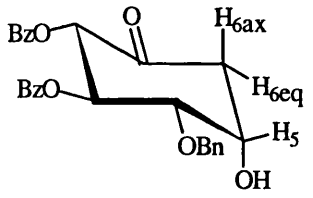
Fig. 2.19 Part of the 400MHz ^{1}H NMR spectrum of (**146**) in $CDCl_3$.

2.4.4 Ferrier Rearrangement of (146)

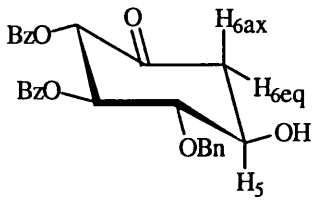
When a stoichiometric amount of mercury (II) trifluoroacetate was added to a solution of (**146**) in aqueous acetone containing 1% acetic acid, the starting material was rapidly consumed, as observed by TLC. However, no clearly defined product was apparent until the addition of sodium chloride. On purification, the expected C-5 epimeric cyclohexanones (**147**) and (**148**) were isolated, in yields of 5% and 50% respectively. The structures were assigned mainly on the basis of ^{1}H NMR and ^{13}C NMR spectroscopy. The isomers were easily distinguishable by comparing the coupling constants of the newly generated axial methylene proton at position 6 (fig. 2.20). In (**147**) this proton experiences a geminal (AB) coupling of 14.5Hz and a vicinal axial-axial coupling of 11.2Hz with the position 5 methine; in (**148**) the geminal coupling (12Hz) is accompanied by the much smaller (vicinal) axial-equatorial coupling of 3.7Hz. In both cases the less shielded position 6 equatorial proton experiences a geminal coupling together with a smaller (equatorial-axial in **147**; equatorial-equatorial in **148**) vicinal coupling, giving essentially the same splitting pattern.

The proportion of minor product (**147**) in this case is much lower than in those reported for the corresponding 2,3,4-tri-*O*-benzyl (3:1) and 2,4-di-*O*-allyl-3-*O*-benzyl (6:1) D-*xylo*- precursors and the 2-*O*-benzyl-3,4-*O*-cyclohexylidene-L-*arabino*- precursor (2:1) (section 2.2.4). It is noteworthy that lower proportions of minor product (if any) have been isolated in all previous examples in which esters, rather than ethers, have been employed at positions 3 and 4. This may indicate a subtle influence of protecting groups on the stereochemical outcome of the mercury (II)-catalysed rearrangement.

A catalytic Ferrier rearrangement of (**146**) using mercury (II) trifluoroacetate increased the overall yield obtained, but did not alter the product ratio. Neither did the palladium (II) chloride reaction,[170,171] which had significantly increased the proportion of the minor product observed in a previous example (table 2.1, section 2.2.2).[171]



(148)



(147)

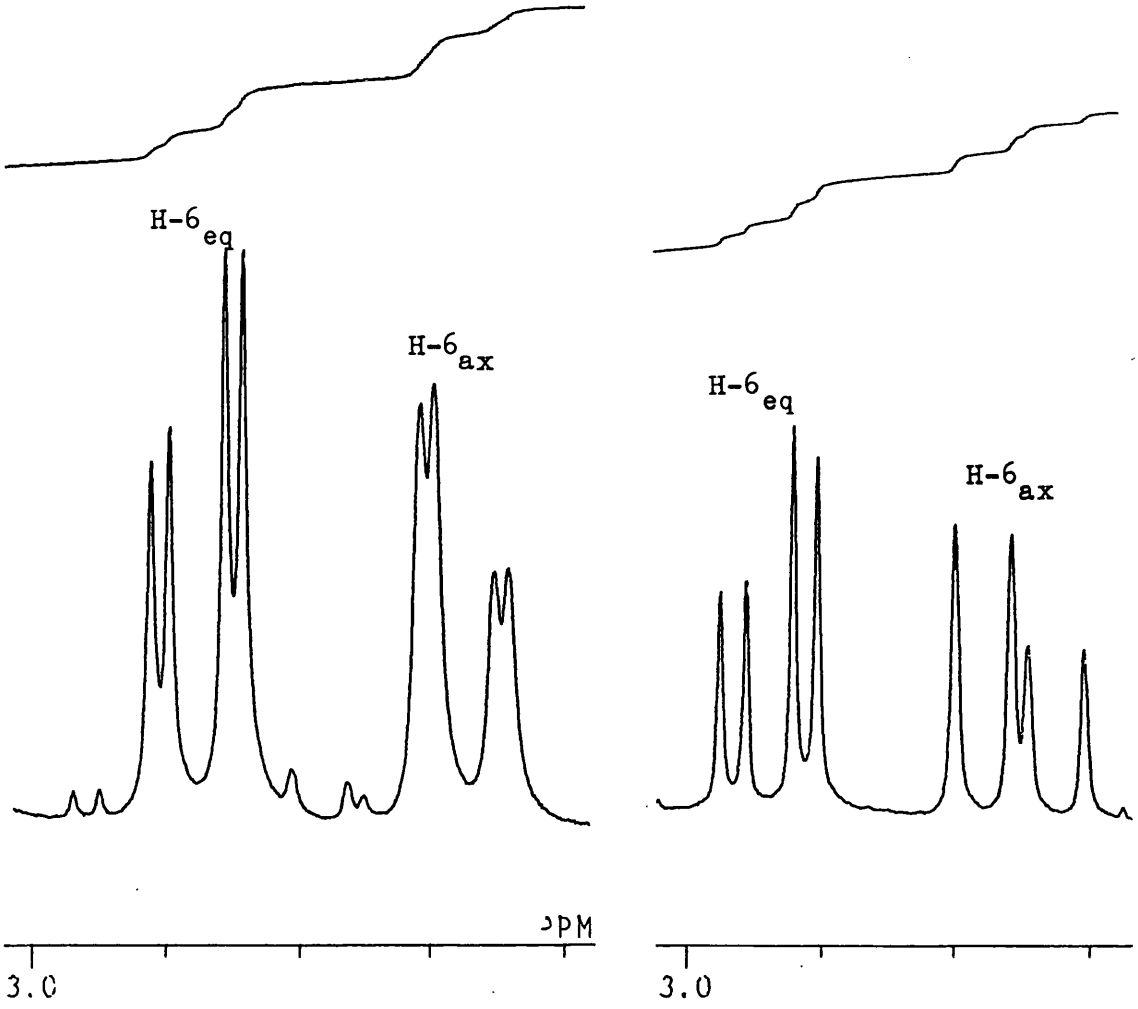
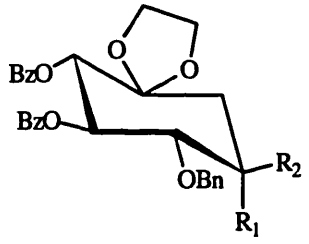


Fig. 2.20 High field regions of the 270MHz ^{1}H NMR spectra of (**148**) and (**147**) in $CDCl_3$ showing coupling of the position 6 methylene protons.

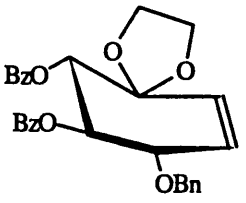
2.4.5 Attempted Inversion of Stereochemistry at position 5 of (148)

Attention now turned to inversion of stereochemistry at position 5 of (**148**); a literature search found no previous attempt at inverting the hydroxyl group at this position of Ferrier products.

Ferrier products readily undergo β-elimination to give β-enones.[154,173,180,189] In order to minimise this side reaction it was decided to protect (**148**) as ethylene acetal (**168**). Ferrier and Haines[173] examined several methods for introducing an ethylene acetal to the related cyclohexanone (**85**) and optimised their yield by reacting (**85**) with ethylene glycol in dioxane-benzene containing a catalytic quantity of H_2SO_4. In the case of (**148**) this method (substituting toluene for benzene) provided 54% of (**168**), but also 33% of the eliminated product (**169**).



(**168**) R_1=OH; R_2=H
(**170**) R_1=H; R_2=$OCOCH_3$



(**169**)

Mitsunobu conditions[234] [DEAD-Ph_3P or 1,1'-(azodicarbonyl)-dipiperidine-Bu_3P] failed to give any reaction, in keeping with previous inositol examples.[235–237]

Attempted S_N2 inversions of the 5-*O*-triflate with caesium acetate in DMF,[71] or of the 5-*O*-mesylate with caesium acetate in refluxing toluene in the presence of crown ethers[238] provided only 12–18% of the required acetate (**170**), together with 50–55% of (**169**). The structure of (**170**) was assigned on the basis of its ^1H NMR spectrum (fig. 2.21). The axial position 6 methylene proton presented as a triplet with an axial-axial vicinal coupling equal to the geminal coupling; the methyl group of the acetate presented as a

singlet at 2.01ppm; and the deshielded position 5 methine was observed as a ddd exhibiting two axial-axial couplings and one axial-equatorial coupling.

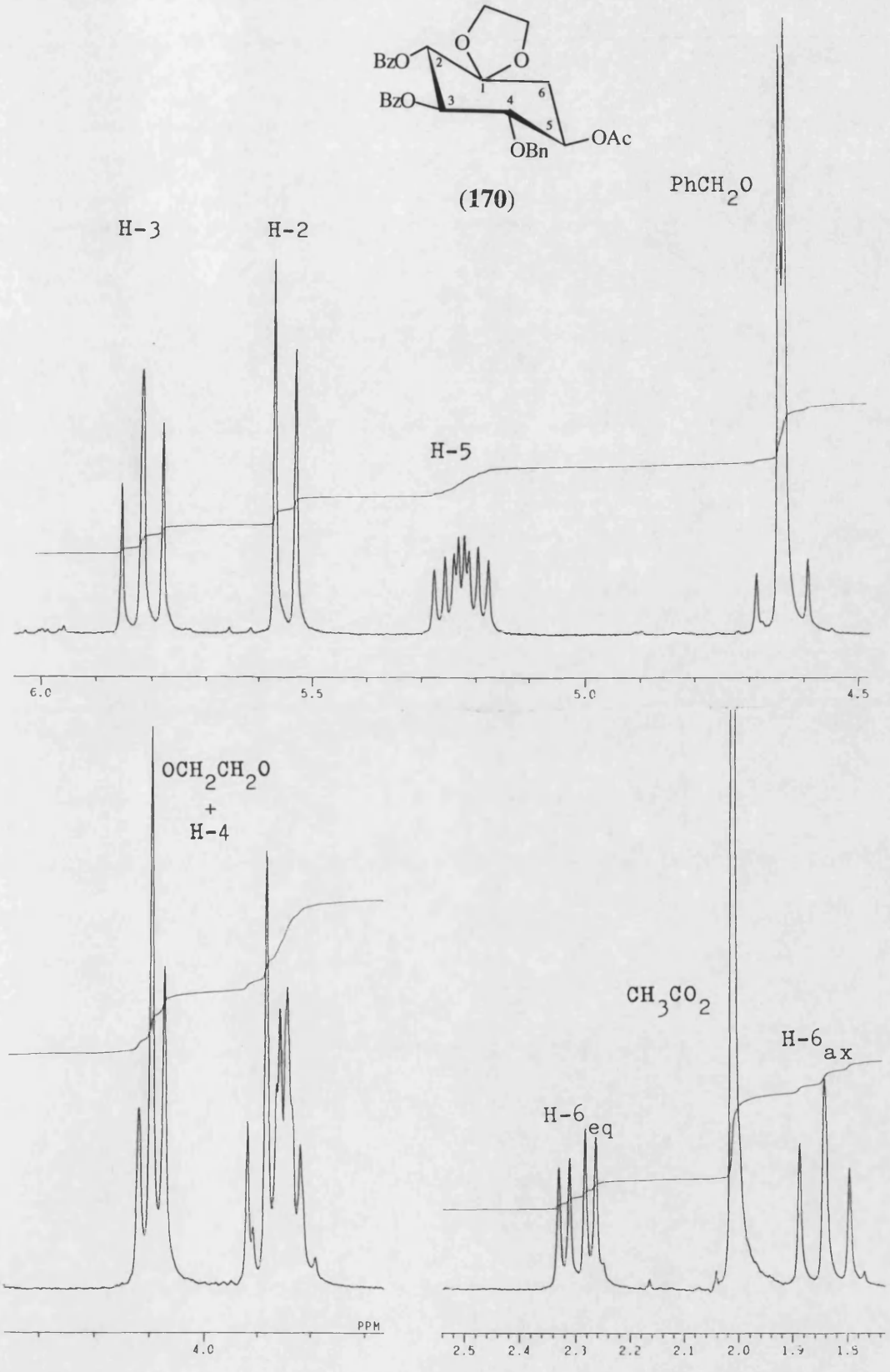
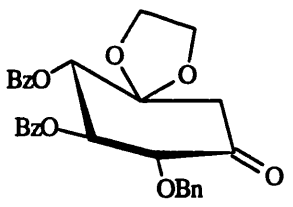


Fig. 2.21 Part of the 400MHz ^{1}H NMR spectrum of (**170**) in $CDCl_3$.

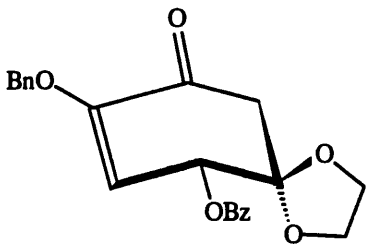
The tendency of (**168**) to eliminate, despite protection of the ketone of (**148**), may be explained by the antiperiplanar arrangement of the axial H-6 proton and the leaving group at C-5.

As the yield of inverted products using the above methodology was unsatisfactory, attention turned to an oxidation-reduction procedure which should avoid the elimination problem. Oxidation of (**168**) using oxalyl chloride and DMSO in dichloromethane ("Swern" oxidation)[239] gave a highly crystalline, strongly dextrorotatory product in high yield which was not, however, the required product (**171**), but enone (**172**), *i.e.* elimination of the benzoate at position 3 of (**168**) had also occurred. The driving force for this elimination is the resultant conjugation, but the mechanism is less obvious. An antiperiplanar arrangement of the axial H-4 proton and the *cis*-C-3-benzoate cannot occur and triethylamine is therefore unlikely to abstract H-4 in the classical E2 mechanism. An alternative possibility is an entirely intramolecular mechanism (fig. 2.22) involving anchimeric assistance from the C-3-*O*-benzoate to give a six-membered ring transition state.

The ^{13}C NMR spectrum of (**172**) was particularly useful in assigning its structure: all six ring carbons exhibited predictable chemical shift values (given in fig. 2.22) and signals behaved as expected in DEPT experiments.

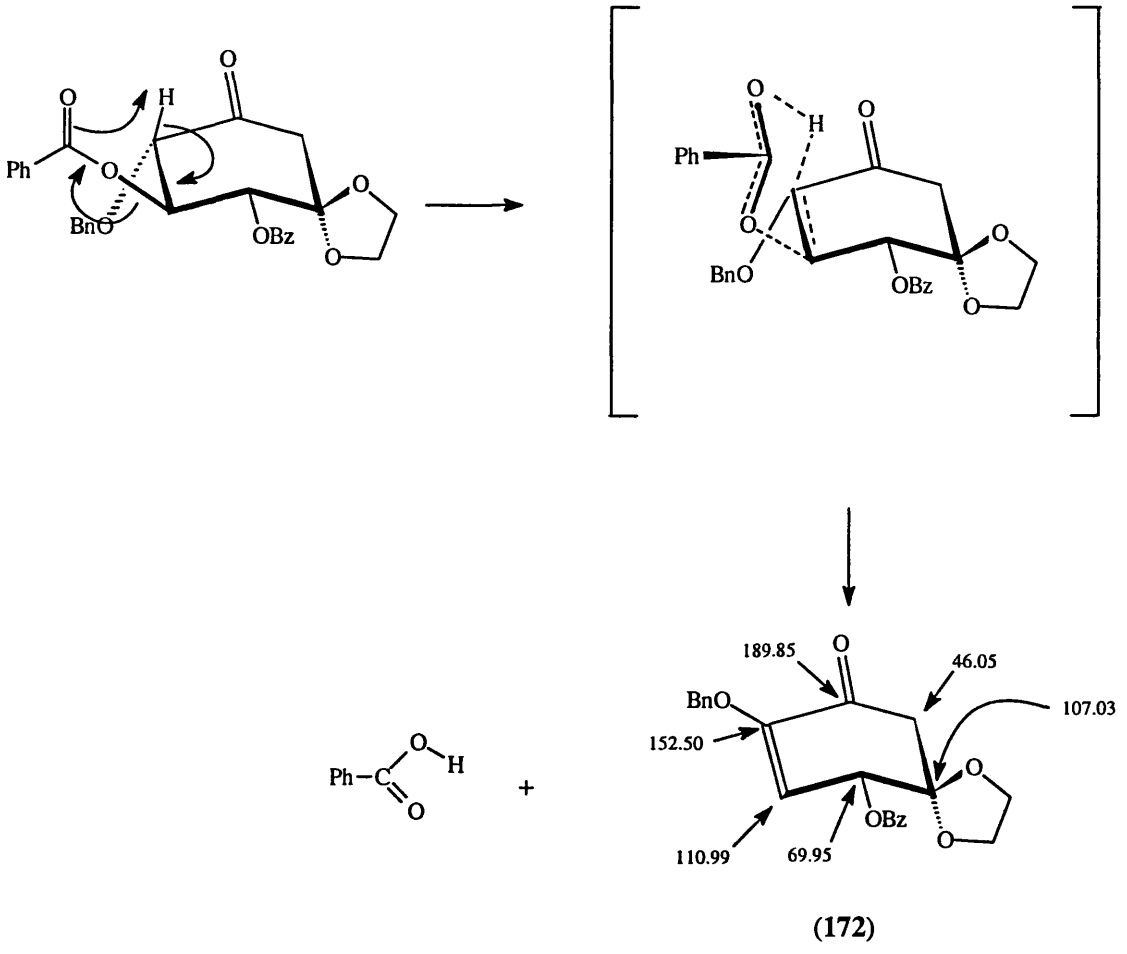


(**171**)



(**172**)

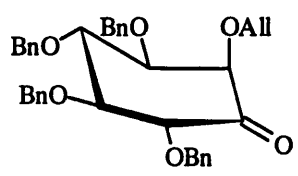
The required ketone (**171**) was eventually obtained by oxidation of (**168**) with pyridinium chlorochromate.[240] Compound (**171**) was obtained in 53% yield by crystallisation from ethanol and further quantities (total 62%) were available by flash chromatography of the mother liquors.



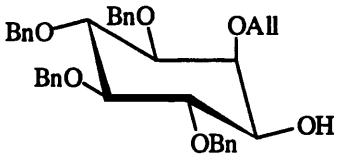
(172)

Fig. 2.22 Possible mechanism for elimination of benzoate during formation of (**172**) and ^{13}C NMR chemical shift values for the cyclohexenone ring of (**172**).

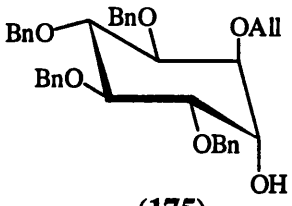
Reduction of (**171**) with sodium borohydride in dioxane or with sodium borohydride-cerium trichloride in methanol gave exclusively the axial alcohol (**168**). Jaramillo and Martín-Lomas[237,241] experienced similar difficulty attempting to reduce the cyclohexanone (**173**) to the *myo*-inositol derivative (**174**). The only reagents which gave (**174**) in appreciable yield (rather than **175**) were (*R*)- and (*S*)-Alpine hydride. Reduction of (**171**) with (*R*)-Alpine hydride at –78°C in THF also gave only (**168**).



(173)



(174)



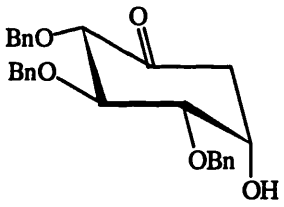
(175)

On building a Dreiding model of (**171**) it became clear that an equatorial approach of the reducing agent (leading to **168**) is much less hindered than an axial approach.

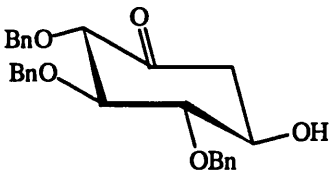
At this stage, inversion attempts were not pursued further, and the minor Ferrier rearrangement product (**147**) was used to prepare the target tetrakisphosphate (**78**).

2.4.6 Preparation of 2-deoxy-Ins(1,3,4,5)P_4

Conversion of (**147**) to the target tetrakisphosphate required reduction of the ketone to the equatorial alcohol, benzoate hydrolysis to provide tetrol (**138**), followed by phosphorylation and complete deblocking.



(94)



(95)

In an earlier study,[164] the 2,3,4-tri-*O*-benzyl Ferrier products (**94**) and (**95**) were reduced with sodium borohydride in dioxane. While (**94**) gave exclusively the axial product, (**95**) was found to give an approximately 1:1 mixture of the axial and equatorial products. Treatment of (**147**) in this way gave D-1,6-di-*O*-benzoyl-5-*O*-benzyl-3-deoxy-*myo*-inositol (**176**) and D-1,2-di-*O*-benzoyl-3-*O*-benzyl-5-deoxy-*scyllo*-inositol (**177**), in a ratio of *ca.* 1:3.3 respectively. The structures of (**176**) and (**177**) were established by considering the coupling constants of the deshielded methine proton vicinal to the newly created hydroxyl group. In (**176**), this signal (H-1) presented as a doublet of doublets

having an axial-axial coupling (to H-6) and an axial-equatorial coupling (to H-2); in (**177**) it experiences two axial-axial couplings and presented as a triplet.

The benzoate esters of (**177**) were smoothly cleaved with methanolic sodium hydroxide to give D-1-*O*-benzyl-3-deoxy-*scyllo*-inositol (**138**) in 86% yield.

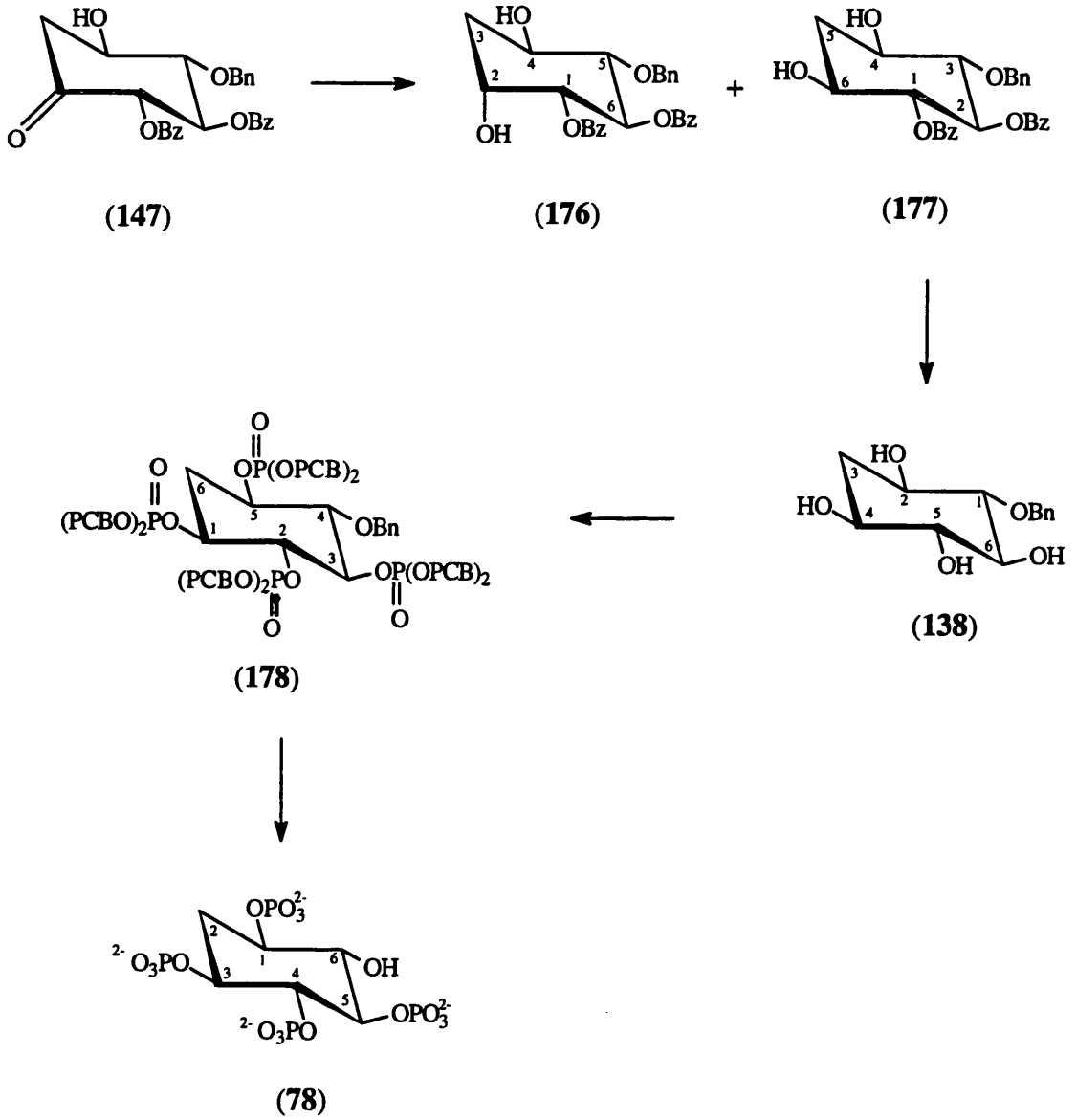


Fig. 2.23 Route to (**78**) from (**147**).

Phosphitylation of (**138**) was carried out at room temperature using the P(III) reagent bis(*p*-chlorobenzyloxy)(diisopropylamino)phosphine[242] with 2 equiv. of phosphitylating reagent per hydroxyl group and 3 equiv. of tetrazole, in a small volume of dry

dichloromethane. After the mixture of phosphitylating reagent and tetrazole had been stirred for 15min, the ^{31}P NMR spectrum indicated a peak at 126.26ppm, corresponding to phosphitylating agent-tetrazolide intermediate. Thirty minutes after addition of the tetrol, the ^{31}P NMR spectrum indicated a complex overlapping pattern of phosphite signals at 139.7–140.0 and 141.5–142.1ppm. The complexity of the spectrum arises from multiple $^5J_{PP}$ coupling.[243] After oxidation with MCPBA and work-up, the ^{31}P NMR spectrum of (**178**) revealed four phosphate signals at –1.59, –1.63, –1.68 and –2.15ppm.

Deblocking of the nine benzyl/*p*-chlorobenzyl protecting groups was achieved using sodium in liquid ammonia.[100] The product was purified using ion-exchange chromatography and phosphate-containing fractions were detected and then combined and the amount of (**178**) quantified using a modification of the Briggs phosphate assay.[244]

The structure of the product was identified as the required tetrakisphosphate (**78**) on the basis of its ^1H-coupled ^{31}P NMR spectrum which showed four doublets, and its ^1H NMR spectrum in D_2O which showed the distinct methylene protons and a triplet corresponding to the ring methine geminal to the sole unphosphorylated alcohol at position 6 (*myo*-inositol numbering). The accurate negative FAB mass spectrum showed a mass consistent with that predicted for $[M-H]^-$.

Preliminary biological evaluation of (**78**) at the Ins(1,4,5)P_3 receptor in SH-SY5Y neuroblastoma cells has been performed by Dr R. A. Wilcox at the University of Leicester. Compound (**78**) was a full agonist with respect to Ca^{2+}-mobilisation, possessing an EC_{50} value of *ca.* 20μM, a value about four-fold higher than that of pure, synthetic D-Ins(1,3,4,5)P_4.

2.5 Subsequent Developments

While this work was in progress, the synthesis of racemic[245] and chiral[246] Ins(1,3,4,5)P_4-3S (**77**) were described. Biological studies[246,247] clearly demonstrated that the D-

enantiomer was a full agonist at the Ins(1,4,5)P_3 receptor, with a potency *ca.* 50-fold lower than Ins(1,4,5)P_3, strongly suggesting that Ins(1,3,4,5)P_4 *can* mobilise intracellular Ca^{2+}.

With the current interest in recently purified Ins(1,3,4,5)P_4-binding proteins, it is hoped that (**78**) will prove a useful ligand in determining the relative importance of the hydroxyl at position 2 of Ins(1,3,4,5)P_4 in ligand-protein binding. These studies, and investigations into the interaction of (**78**) with the enzymes 3-phosphatase, 5-phosphatase and 3-kinase are presently being pursued.

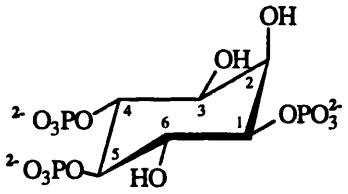
CHAPTER THREE

SYNTHESIS OF A RING-CONTRACTED ANALOGUE OF Ins(1,4,5)P_3

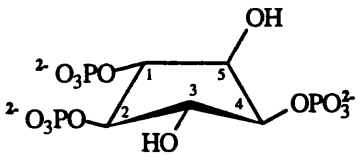
3.1 INTRODUCTION

All reported approaches to structural modification of Ins(1,4,5)P_3 which produced agonists (see chapter 1) at the start of this work had focused upon modifications at phosphorus or hydroxyl group deletion, reorientation, alkylation or replacement with isosteres and other groups in the *six-membered* ring. Despite numerous single and multiple modifications, the fundamental requirement of a six-membered ring had not been addressed. Even in the adenophostins (discussed in chapter five), which differ in many respects from Ins(1,4,5)P_3, the important 3,4-bisphosphate / 2-hydroxyl triad, analogous to the 4,5-bisphosphate / 6-hydroxyl arrangement of Ins(1,4,5)P_3, is contained within the six-membered pyranoside ring.

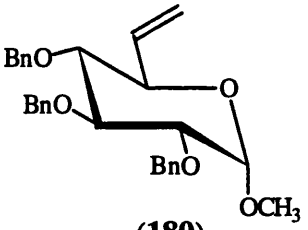
Since many studies demonstrated that positions 2 and 3 of Ins(1,4,5)P_3 are tolerant to extensive modification, it was reasoned that a contracted structure such as (**179**), obtained essentially by deletion of the 2-position carbon of Ins(1,4,5)P_3 with its associated hydroxyl group, should also fulfil the recognition requirements of the Ins(1,4,5)P_3 receptor.



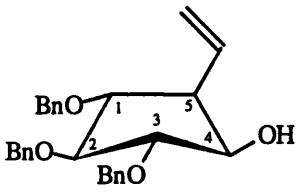
Ins(1,4,5)P_3



(**179**)



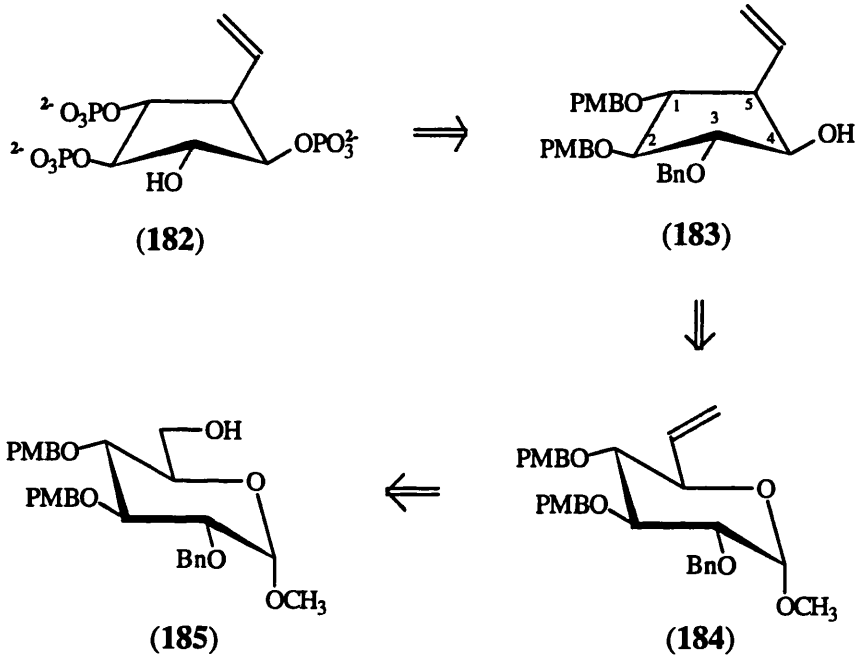
(**180**)



(**181**)

Many methods for converting carbohydrates into cyclopentane derivatives have been described,[153] but the disclosure by Ito *et al.*[248] that treatment of methyl 2,3,4-tri-*O*-benzyl-6,7-dideoxy-α-D-*gluco*-hex-6-enopyranoside(1,5) (**180**) with zirconocene

("Cp_2Zr") followed by boron trifluoride etherate produced vinylcyclopentane (**181**) (see ref. 249 for a review) was of particular interest, as positions 1,2,3 and 4 of (**181**) possess the same relative stereochemistry as the equivalent positions in (**179**), and therefore as positions 4,5,6 and 1 respectively in Ins(1,4,5)P_3. It was decided to apply this methodology in an attempt to prepare (1*R*, 2*R*, 3*S*, 4*R*, 5*S*)-3-hydroxy-1,2,4-trisphospho-5-vinylcyclopentane (**182**; fig. 3.1), an analogue of (**179**) which would allow the viability of cyclopentane-based mimics to be assessed.



(**182**) (**183**) (**185**) (**184**)

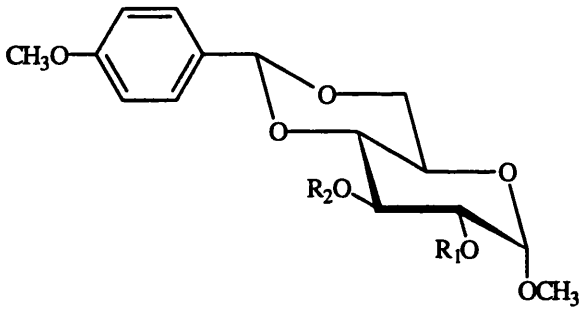
Fig. 3.1 Retrosynthetic analysis showing a potential route to a cyclopentane-based Ins(1,4,5)P_3 analogue.

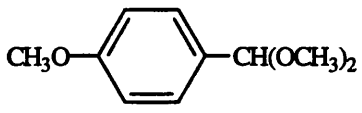
Trisphosphate (**182**) would require a ring-contracted intermediate in which the protecting groups at positions 1 and 2 are orthogonal to that at position 3, to allow selective removal to provide the appropriate triol for phosphorylation. It was decided to employ *p*-methoxybenzyl ethers at positions 1 and 2, and a benzyl ether at position 3 (*i.e.* giving intermediate **183**), as this combination was as close as possible to the tribenzyl arrangement used by Ito *et al.*[248] The carbohydrate precursor to (**183**) is heptoside (**184**). The 5-vinyl moiety of (**184**) would be introduced by successive Swern oxidation and Wittig methylenation[250] of methyl 2-*O*-benzyl-3,4-di-*O*-(*p*-

methoxybenzyl)-α-D-glucopyranoside (**185**). As part of a collaborative effort to produce trisphosphate (**182**), the author examined methods to prepare primary alcohol (**185**).

3.2 Preparation of Methyl 2-*O*-benzyl-3,4-di-*O*-(*p*-methoxybenzyl)-α-D-glucopyranoside (185)

p-Methoxybenzylidene acetals have the property that either of the acetal C—O bonds may be selectively cleaved to furnish a *p*-methoxybenzyl ether.[251] The direction of cleavage depends upon steric and electronic factors as well as upon the choice of cleavage reagent.[251] Various methods[252,253] have been used to cleave the acetal of 2,3-disubstituted derivatives of methyl 4,6-*O*-(*p*-methoxybenzylidene)-α-D-glucopyranoside (**186**) resulting in selective formation of 4-*O*-(*p*-methoxybenzyl) ethers. It was therefore decided to attempt to benzylate (**186**) selectively at position 2 using similar methodology to that developed for the benzylidene derivative (**150**) in chapter 2. After *p*-methoxybenzylation of the hydroxyl group at position 3, reductive cleavage of the acetal should give the target intermediate (**185**).





(**187**)

(**186**) R_1=R_2=H
(**188**) R_1=Bn; R_2=H
(**189**) R_1=H; R_2=Bn
(**190**) R_1=Bn; R_2=PMB
(**191**) R_1=R_2=Bn
(**197**) R_1=R_2=CH_3

p-Methoxybenzaldehyde dimethyl acetal (**187**) was prepared by a slight modification of the method of Johansson and Samuelsson.[252] Thus, a mixture of *p*-methoxybenzaldehyde, trimethyl orthoformate and PTSA was stirred at room temperature. Carrying out the reaction at reduced pressure was found to remove methyl

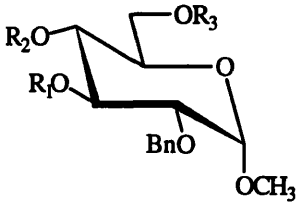
formate (b.p. 31.5°C at 760mmHg[254]) more efficiently, thereby driving the reaction to completion. Compound (**187**) was purified by distillation *in vacuo* and was greater than 99% pure by ^{1}H NMR spectroscopy.

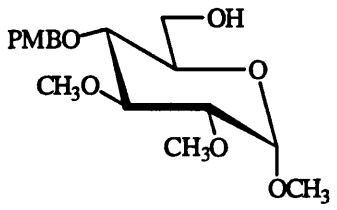
The known[252] (**186**) was prepared in an analogous procedure to (**150**), *i.e.* by reaction of methyl α-D-glucopyranoside with (**187**) at 70°C in DMF containing a catalytic quantity of PTSA, with continuous removal of methanol. Compound (**186**) required about six to eight times more boiling 2%^{w}/$_{v}$ aqueous sodium hydrogen carbonate solution to dissolve it during recrystallisation than (**150**).

Benzylation of (**186**) with dibutyltin oxide and benzyl bromide in acetonitrile in the presence of tetrabutylammonium iodide and 4Å molecular sieves gave a major and a minor product by TLC. These were easily separated by column chromatography and were identified as the 2-benzyl ether (**188**) (48%) and the 3-benzyl isomer (**189**) (11%). The structure of (**188**) was established from its ^{1}H NMR spectrum in $CDCl_3$: coupling of the methine at position 3 to the hydroxyl proton was observed, which disappeared on D_2O exchange. The OH stretching observed in the IR spectra of (**188**) and (**189**) conformed to the generalisations described in chapter 2; in addition, the hydroxyl proton of (**189**) exhibited the characteristically large coupling constant (*J* 7.3Hz) in the ^{1}H NMR spectrum in $CDCl_3$.

The major product (**188**) was smoothly *p*-methoxybenzylated using sodium hydride and *p*-methoxybenzyl chloride in DMF at room temperature, to furnish (**190**). During the work-up it was necessary to use chloroform as extracting solvent because (**190**) was not sufficiently soluble in other solvents.

With (**190**) in hand, attention turned to cleavage of the acetal. Samuelsson and Johansson[252] treated methyl 2,3-di-*O*-benzyl-4,6-*O*-(*p*-methoxybenzylidene)-α-D-glucopyranoside (**191**) with sodium cyanoborohydride and trimethylsilyl chloride in acetonitrile, and obtained the 4-*O*-substituted *p*-methoxybenzyl ether (**192**) in 76% yield, together with 13% of the 6-substituted isomer (**193**).





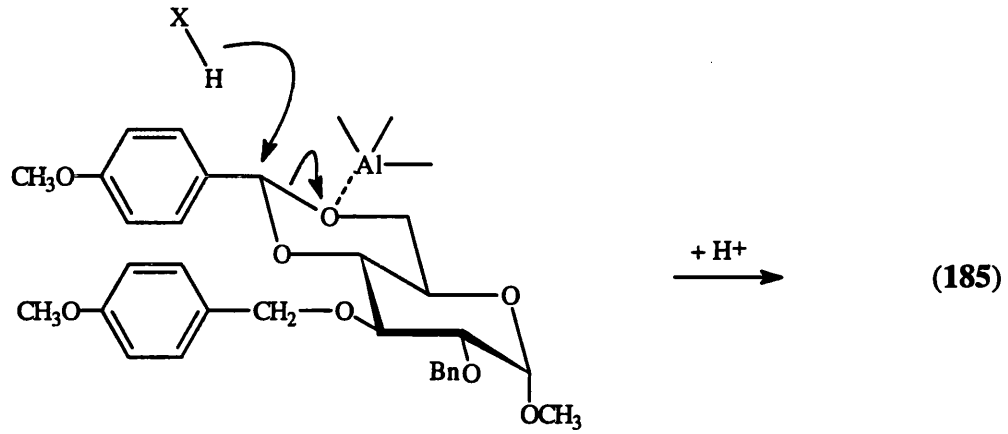
(**192**) R_1=Bn; R_2=PMB; R_3=H
(**193**) R_1=Bn; R_2=H; R_3=PMB
(**194**) R_1=R_3=PMB; R_2=H
(**195**) R_1=R_3=PMB; R_2=Bz
(**196**) R_1=R_2=PMB; R_3=Bz

(**198**)

When (**190**) was treated with these reagents, the chromatographically separable methyl 2-*O*-benzyl-3,6-di-*O*-(*p*-methoxybenzyl)-α-D-glucopyranoside (**194**) and the required 4-substituted isomer (**185**) were obtained in a ratio of *ca.* 1:1.7 . The structures of (**194**) and (**185**) were established by comparison of chemical shifts of their position 6 carbon atoms (identifiable by 135DEPT experiments), as observed in their ^{13}C NMR spectra in $CDCl_3$. The C-6 of (**194**) resonates at lower field (69.18ppm) than that of (**185**) (61.84ppm) due to the α-effect of alkylation.[255] These assigned structures were confirmed by preparation of benzoate esters (**195**) and (**196**). The ^1H NMR spectrum of (**195**) displayed a deshielded triplet at 5.28ppm, corresponding to the position 4 methine; the position 6 methylene protons of (**196**) were deshielded to 4.46–4.97ppm.

As selectivity of cleavage using the above method was disappointing, alternative conditions were explored. Joniak *et al.*[253] used $LiAlH_4$-$AlCl_3$ to convert methyl 4,6-*O*-(*p*-methoxybenzylidene)-2,3-di-*O*-methyl-α-D-glucopyranoside (**197**) exclusively to the 4-*O*-(*p*-methoxybenzyl) ether (**198**). Reaction of (**190**) with $LiAlH_4$-$AlCl_3$ in refluxing THF gave exclusively the required product (**185**) in 73% yield. This result supports the suggestion[253] that a substituent at position 3 of the glucopyranoside ring hinders access of the reagent to the oxygen lone pair at position 4, thereby directing exclusive coordination to the oxygen at position 6 (fig. 3.2). Cleavage of the acetal C—O-6 bond then results in exclusive formation of the 4-substituted ether. Similar effects have been reported for the cleavage of related 4,6-*O*-benzylidene acetals in 3-substituted glucose derivatives.[257] Why the electrophile in the Me_3SiCl-$NaCNBH_3$ experiment should

coordinate to the position 4 acetal oxygen more easily than does the $LiAlH_4$-$AlCl_3$ reagent is not clear.



(185)

(190)

Fig 3.2 Cleavage of the 4,6-*O*-(*p*-methoxybenzylidene) acetal of (**190**) using $LiAlH_4$ -$AlCl_3$. The active species is believed to be AlH_2Cl (ref. 256).

The rest of the synthetic route to (**182**) (fig. 3.3) was completed by another worker, and proceeded smoothly.[258] Of particular interest was the ring-contraction step, which gave a small amount of (**199**) in addition to the required (**183**). An equivalent minor product from the tribenzyl *gluco* precusor (**180**) had not been reported by Ito *et al.*[248] The relative stereochemistry of (**199**) and (**183**) was determined by 2D NOESY and NOE difference spectroscopy.

***Biological data*:** Trisphosphate (**182**) was examined for heparin-sensitive Ca^{2+} mobilising activity at the platelet $Ins(1,4,5)P_3$ receptor using fluorescence techniques, and also using saponin-permeabilised platelets loaded with $^{45}Ca^{2+}$. It was found to be a full agonist [EC_{50} 23μM; *cf.* $Ins(1,4,5)P_3$ 0.2μM], confirming its functional recognition by this receptor. These results demonstrate that $Ins(1,4,5)P_3$ receptor mediated Ca^{2+} mobilisation does not necessarily require a cyclohexyl (or equivalent) structural motif. A smaller ring phosphate which retains crucial recognition elements of $Ins(1,4,5)P_3$, *i.e.*

three appropriately orientated phosphates and a pseudo-6-hydroxyl group, can still exhibit agonistic activity.

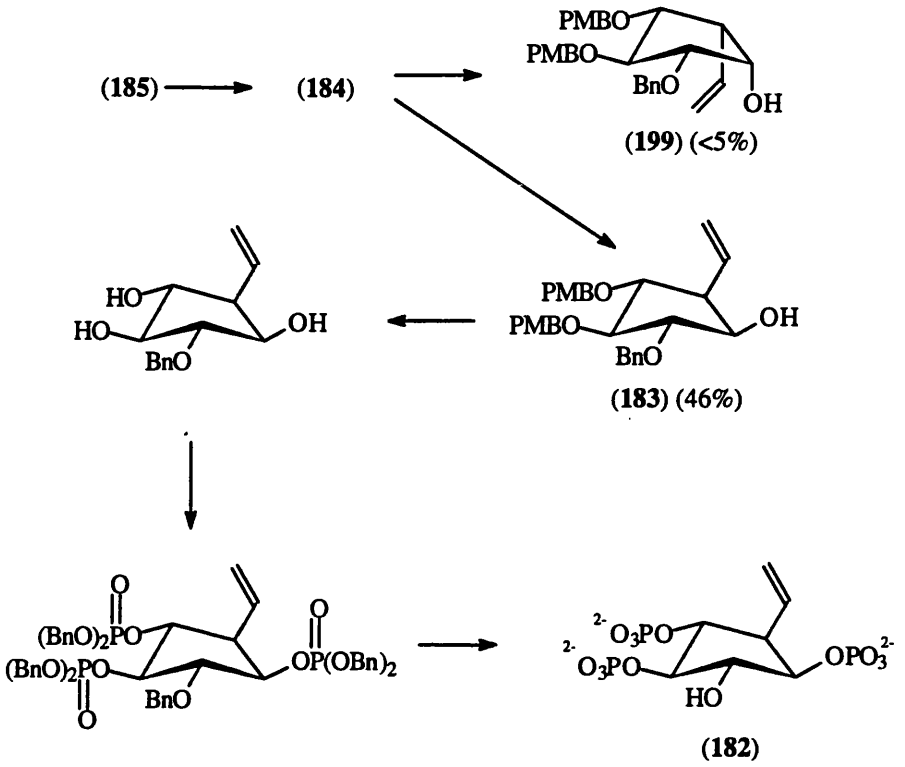
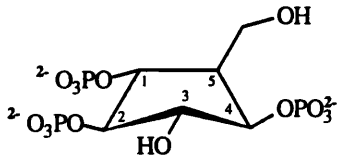


Fig. 3.3 Synthesis of (**182**) from (**185**).

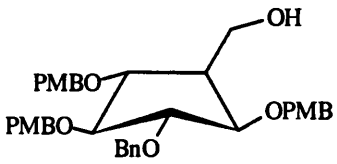
3.3 Preparation of (1*R*, 2*R*, 3*S*, 4*R*, 5*S*)-3-Hydroxy-5-hydroxymethyl-1,2,4-trisphosphocyclopentane (200).

Noting that DL-3-*O*-methyl-Ins(1,4,5)P_3 and DL-3-*O*-ethyl-Ins(1,4,5)P_3 both have EC_{50} values of greater than 100μM in SH-SY5Y neuroblastoma cells [*cf.* Ins(1,4,5)P_3 0.18μM],[259] it seemed reasonable that replacement of the hydrophobic vinyl group of (**182**) with a hydroxyl-containing side chain should markedly increase its potency. An obvious target was (**200**), in which the vinyl substituent is replaced by hydroxymethyl. Initially it was intended to prepare (**200**) by *p*-methoxybenzylation of (**183**) followed by treatment with OsO_4-$NaIO_4$ and reduction of the intermediate aldehyde to give (**201**). Benzylation of the primary alcohol followed by removal of *p*-methoxybenzyl ethers

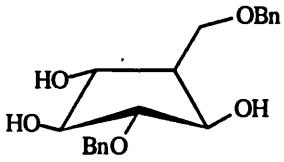
would furnish triol (**202**), which on phosphorylation and deprotection would provide (**200**).



(**200**)



(**201**)



(**202**)

However, a recent report by Chénedé *et al.*[260] offered the possibility of a simpler route. These workers treated aldehyde (**203**; fig. 3.4) with samarium (II) iodide in the presence of *t*-butanol and HMPA, and obtained cyclopentane (**204**). The structure of (**204**) was established by conversion to known compounds, and a mechanism for the ring contraction has been proposed.[260] It was decided to attempt this rearrangement on aldehyde (**205**), which should give (**206**). Benzylation of the primary hydroxyl group and acidic hydrolysis would furnish triol (**202**), which could be elaborated to (**200**).

Aldehyde (**205**) was prepared by Swern oxidation of (**185**). After column chromatography, some O–H stretching was apparent in the IR spectrum of the product in addition to the expected C=O stretching. As azeotropic drying abolished this O–H stretching, it was assumed to arise from hydration of the aldehyde rather than from contamination by starting material. Although sufficiently stable to allow full characterisation (which had not been carried out previously), (**205**) gradually rehydrated after standing in air for several days, and was therefore best prepared freshly.

Treatment of (**205**) with samarium (II) iodide in THF in the presence of *t*-butanol and HMPA with rigorous exclusion of air and moisture gave two products after 1 h, as judged by TLC. These were identified as alcohol (**185**; 19%), arising from reduction of the aldehyde, and (**206**; 37%).

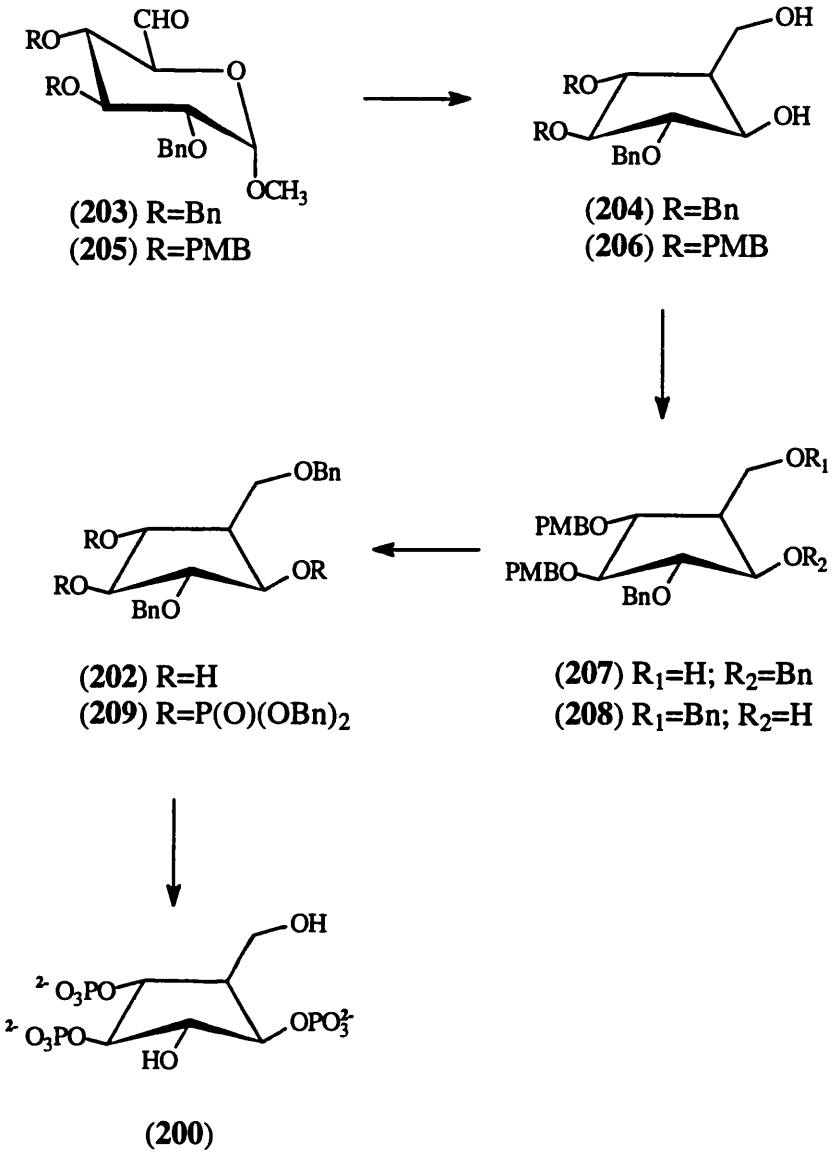


Fig. 3.4 Route to cyclopentane-based trisphosphate (**200**).

The structure of (**206**) was assigned as follows: the negative FAB mass spectrum showed a peak at *m/z* 493, corresponding to $(M-1)^-$. Characteristic carbohydrate signals were absent from both ^1H and ^{13}C NMR spectra. The ^{13}C NMR spectrum contained *inter alia* a methine signal at 46.68ppm, assigned as C-5 and a methylene signal at 60.93ppm, assigned as the hydroxymethyl carbon. The ^1H NMR spectrum exhibited a high-field one-proton signal at 2.24ppm corresponding to the methine at position 5 in addition to other expected signals. Part of the ^1H 2D COSY spectrum of (**206**) is given in fig 3.5. Admittedly, the stereochemistry of (**206**) cannot be unambiguously assigned on the basis of these data. An NOE study would not be conclusive because there is only

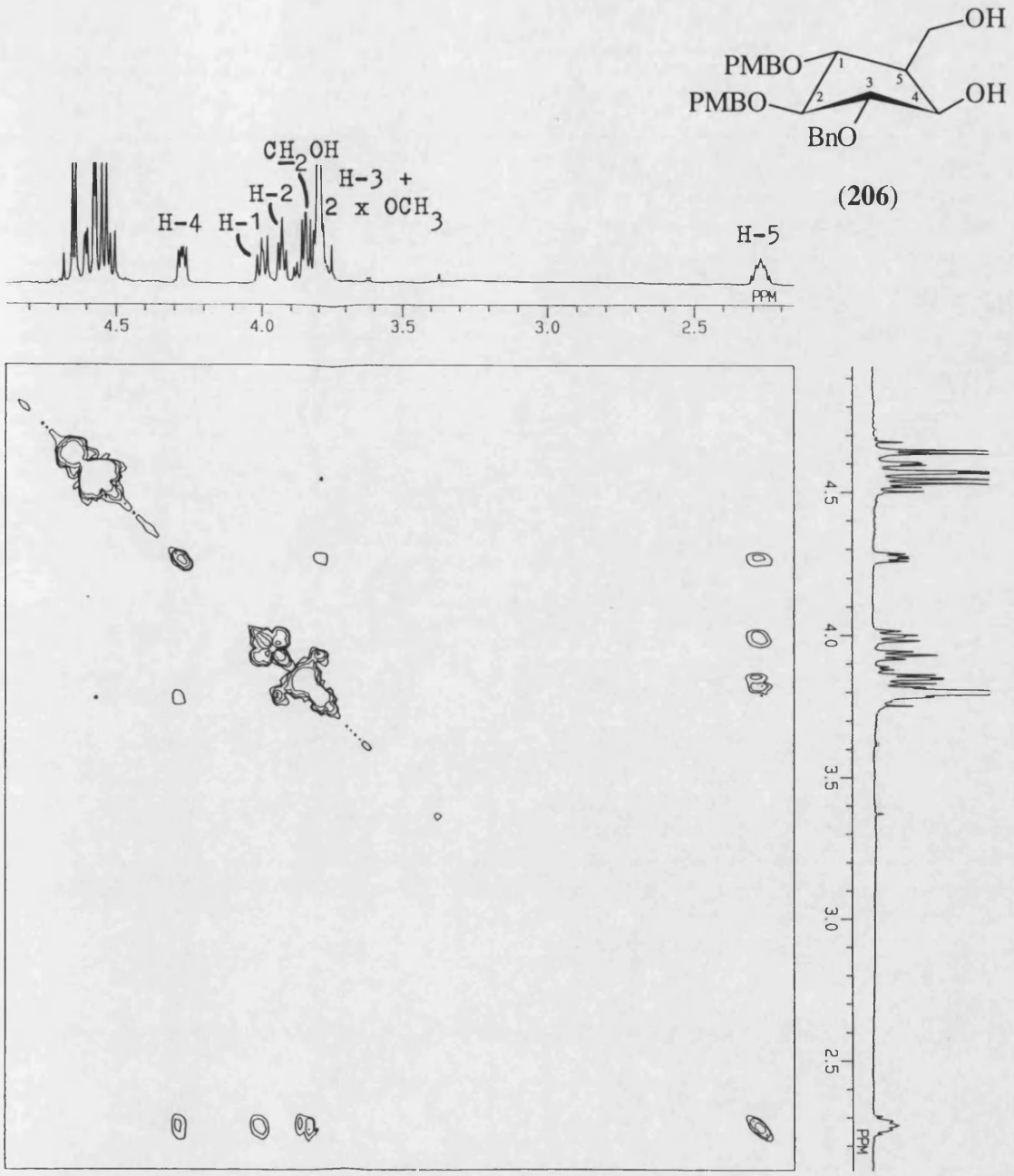
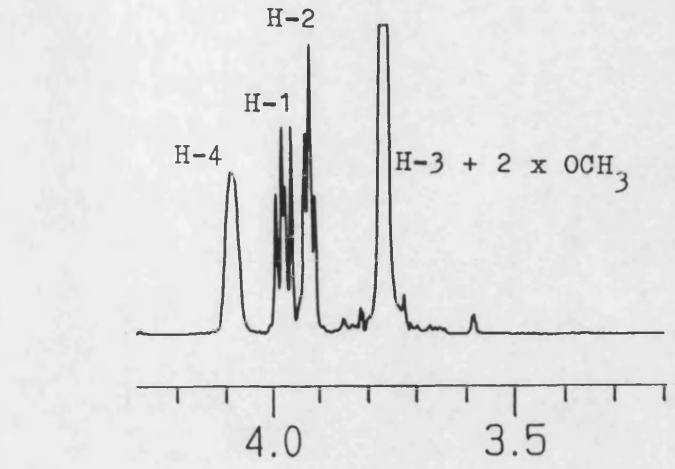
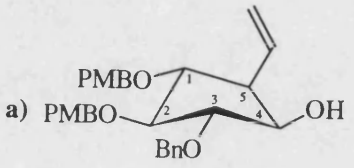


Fig. 3.5 Part of the ^{1}H 2D COSY NMR spectrum of (**206**) in $CDCl_3$.

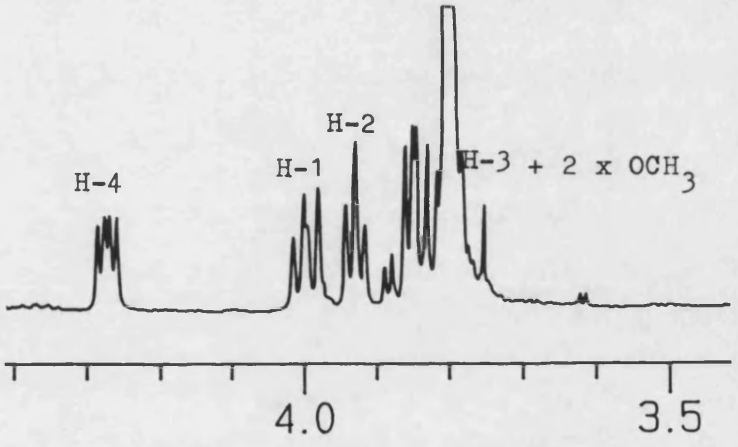
one isomer and the absence of an NOE effect does not exclude a particular stereochemical arrangement. However, the ^{1}H NMR spectrum of (**206**; fig. 3.6b) was compared with those of the two isomers (**183**; fig. 3.6a) and (**199**; fig. 3.6c) obtained from the zirconium-mediated contraction.[258] The similar relative chemical shift values (and splitting patterns) of H-4, H-1 and H-2 of (**206**) and (**183**) is apparent, as is the dissimilarity between these positions of (**206**) and (**199**). The structure of (**206**) was

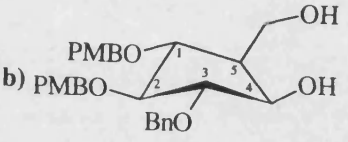
therefore assigned as shown, consistent with the results of Chénedé *et al.*[260] It is intended eventually to confirm this assignment by benzylation of (**183**) and conversion to the hydroxymethyl derivative (**207**; *vide infra*) as outlined above.



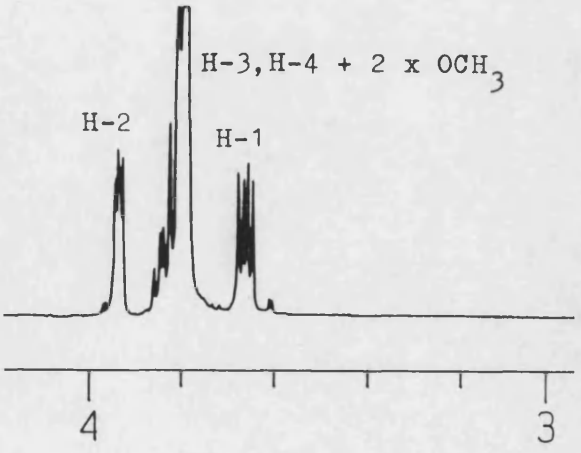


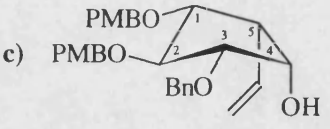
(**183**)





(**206**)





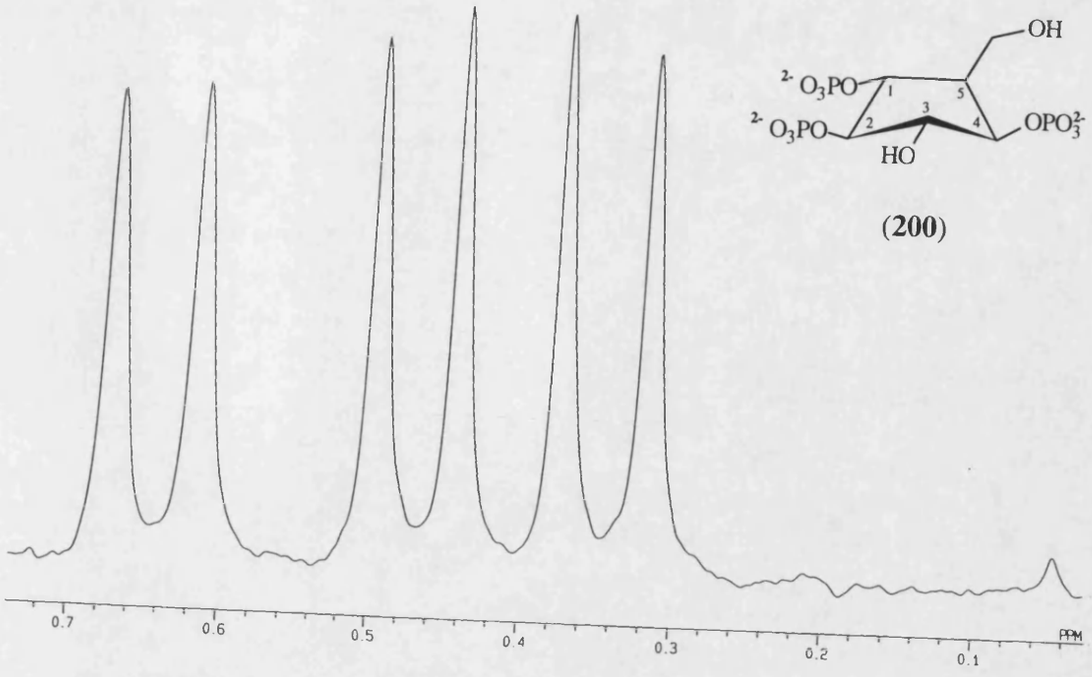
(**199**)

Fig. 3.6 Part of the ^{1}H NMR spectra of a) (**183**), b) (**206**) and c) (**199**) in $CDCl_3$.

Benzylation of (**206**) at 0°C with 1 equiv. sodium hydride and 1.1 equiv. benzyl bromide in dry DMF gave a mixture of (**207**) and (**208**) in a ratio of 3:2 respectively, together with unreacted starting material. The structures of (**207**) and (**208**) were distinguished between by comparison of chemical shifts of the methylene carbon atoms of the C-5 side chain in the ^{13}C NMR spectra: the α-effect of alkylation causes this signal in the spectrum of (**208**) (67.98ppm) to be deshielded relative to that of (**207**) (60.95ppm).

Acidic hydrolysis of (**208**) with 2:1 ethanol–1M aqueous HCl provided triol (**202**) in 72% yield. Phosphitylation of (**202**) with tetrazole-activated bis(benzyloxy)(diisopropyl-amino)phosphine in dichloromethane provided a trisphosphite intermediate, the ^{31}P NMR spectrum of which exhibited a singlet at 140.3ppm and an AB system at 140.2 and 139.9ppm with a characteristically[258] large $^{5}J_{PP}$ coupling constant of 7.0Hz. Oxidation of this intermediate with MCPBA provided the fully protected intermediate (**209**). Compound (**209**) contained some impurities which could not be entirely removed by column chromatography with various eluents. As the impurities did not contain phosphorus, as judged by ^{31}P NMR spectroscopy, the crude material was deprotected using sodium in liquid ammonia. The target trisphosphate (**200**) was obtained pure as its triethylammonium salt after ion-exchange chromatography and was quantified by Briggs phosphate assay.

The ^{1}H-coupled ^{31}P NMR spectrum of (**200**) (fig. 3.7) comprised three doublets, confirming the presence of three ring phosphates. The ^{1}H NMR spectrum of (**200**) in D_2O (fig. 3.8) contained a high-field quintet at 2.29ppm corresponding to the methine at position 5; an ABX system at 3.65 and 3.71ppm corresponding to the methylene protons; a triplet at 3.94ppm corresponding to the methine at position 3; and a deshielded three-proton multiplet corresponding to the ring methines geminal to phosphate esters.



(**200**)

Fig. 3.7 162MHz ^{1}H-coupled ^{31}P NMR spectrum of the triethylammonium salt of (**200**) in D_2O, pH *ca.* 4.

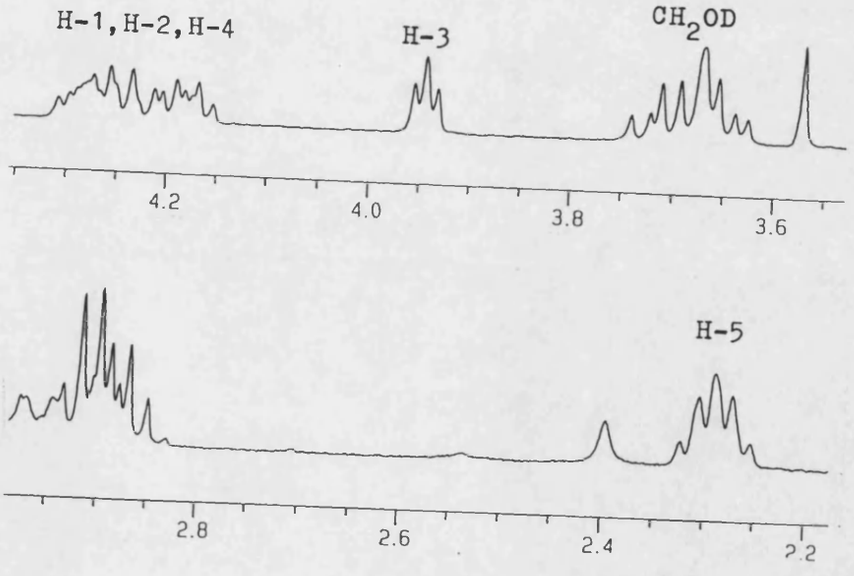


Fig. 3.8 Part of the 400MHz ^{31}P -coupled ^{1}H NMR spectrum of the triethylammonium salt of (**200**) in D_2O, pH *ca.* 4.

The biological properties of (**200**) are being evaluated.

In conclusion, two recently reported carbohydrate ring-contraction methods have been successfully applied to prepare chiral cyclopentane-based Ins(1,4,5)P_3 analogues.

CHAPTER FOUR

ATTEMPTED PREPARATION OF A VERSATILE CHIRAL INTERMEDIATE FOR SYNTHESIS OF ANALOGUES OF Ins(1,4,5)P_3

4.1 Introduction

There are several problems inherent to the synthesis of inositol polyphosphates starting from *myo*-inositol. First, selective protection of the hydroxyl groups is necessary. Second, as *myo*-inositol possesses a plane of symmetry through C-2 and C-5 (fig. 4.1), its protection often leads to racemic mixtures of compounds; as only one inositol polyphosphate enantiomer is usually active, racemic precursors need to be resolved by conversion to a pair of diastereoisomers which may be separable by crystallisation or column chromatography. Third, efficient phosphorylation of the appropriate polyol is required. Fourth, efficient deprotection without phosphate migration must be achieved and fifth, the final target polyphosphate must be purified.

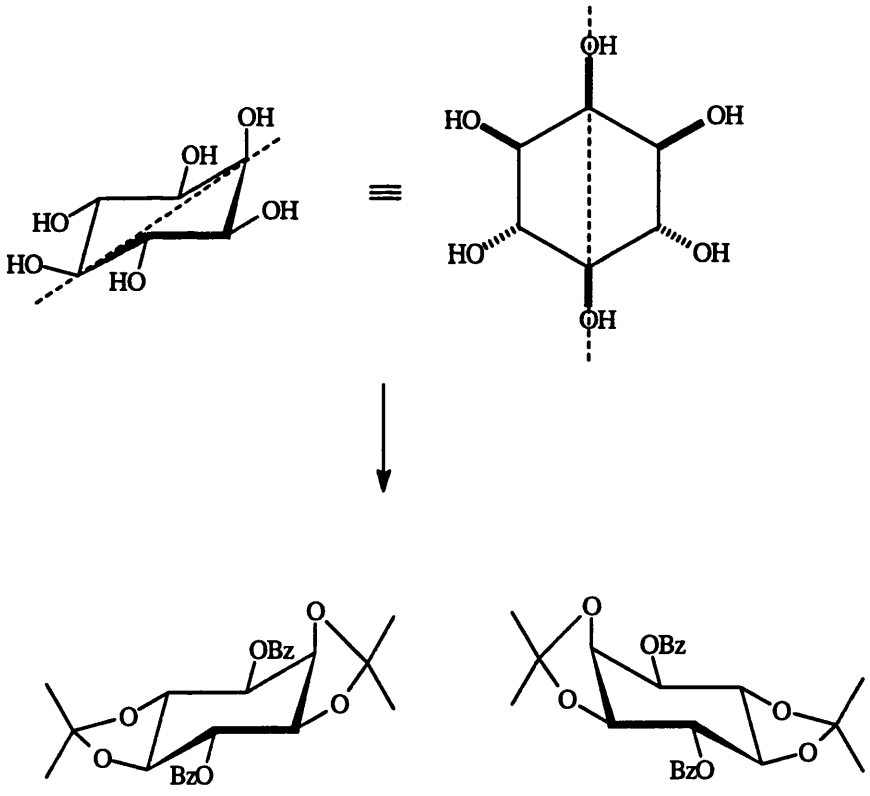
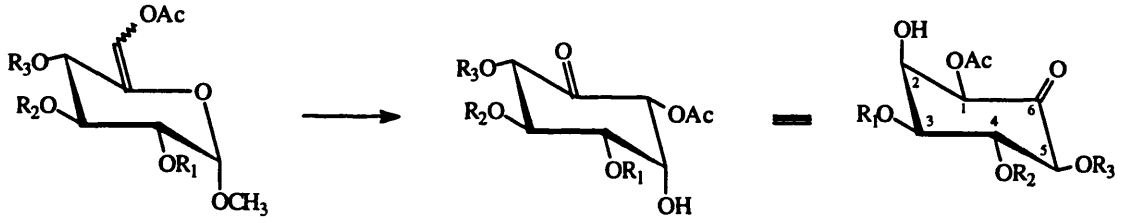


Fig. 4.1 The plane of symmetry in *myo*-inositol oftens leads to racemic mixtures of subsequently protected compounds.

The last three problems have generally been adequately addressed during the last ten years.[11] However, the efficient preparation of a versatile, chiral inositol intermediate

which would allow many target polyphosphates to be prepared has been less satisfactorily achieved, and an attempt to prepare such an intermediate is described in this chapter.

Conversion of a selectively protected carbohydrate to an assymmetrically protected inositol would obviate the need for resolution. In particular, Bender and Budhu's modification of the Ferrier rearrangement[167] (section 2.2.4), in which enol acetates (**129ab**; fig. 4.2) are transformed into D-*myo*-inos-6-oses (**130**), is particularly elegant, as the Ferrier step directly introduces a selectively protected hydroxyl group (as an acetate ester) at position 1. The ketone of compounds (**130**) can be stereoselectively reduced to give *myo*-inositol derivatives in over 90% yield using sodium triacetoxyborohydride,[167] and the resulting two hydroxyl groups have been benzyloxymethylated in the presence of the acetate ester.[191]



(**129ab**) (**130**)

Fig. 4.2 Direct synthesis of D-*myo*-inos-6-oses from D-*xylo*-hex-5-enopyranosides.

Positions 2, 3 and 4 of methyl α-D-glucopyranoside (**142**) possess identical relative stereochemistry to positions 3, 4 and 5 respectively of D-*myo*-inositol derivatives. Therefore, if R_1 in (**129ab**) was orthogonal with respect to all other groups used, the scheme outlined in fig. 4.2 potentially allows the preparation of a chiral D-*myo*-inositol intermediate in which positions 1, 3 and the 4, 5-diol are all independently accessible.

Choice of protecting groups is important. Allyl ethers were felt to be inappropriate in this case as the Ferrier step involves binding of mercury (II) ions to a double bond.* However, *p*-methoxybenzyl ethers may be removed in the presence of, *inter alia*,

* A recent report (ref. 61) has shown that allyl ethers are unaffected by catalytic Ferrier conditions; whether they are stable to prolonged exposure to the stoichiometric quantities of mercury (II) ions used in the modified route remains to be established.

benzyloxymethyl ethers, ketones, acetate esters and isopropylidene ketals.[261] Introduction of a PMB group to position 2 of (**142**) is therefore logical.

Positions 4 and 5 of *myo*-inositol intermediates are frequently protected with an isopropylidene ketal;[11] formation of a 3,4-*O*-isopropylidene in this case would give (**210**; fig. 4.3). The product of a Ferrier rearrangement on the corresponding enol acetate would be D-1-*O*-acetyl-4,5-*O*-isopropylidene-3-*O*-(*p*-methoxybenzyl)-*myo*-inos-6-ose (**211**). Reduction of (**211**) would give the corresponding *myo*-inositol derivative (**212**). In addition, protection of the hydroxyl group of (**211**) and elaboration of the ketone potentially allows chiral analogues of both $Ins(1,4,5)P_3$ and $Ins(1,3,4,5)P_4$ modified at position 6 to be synthesised.

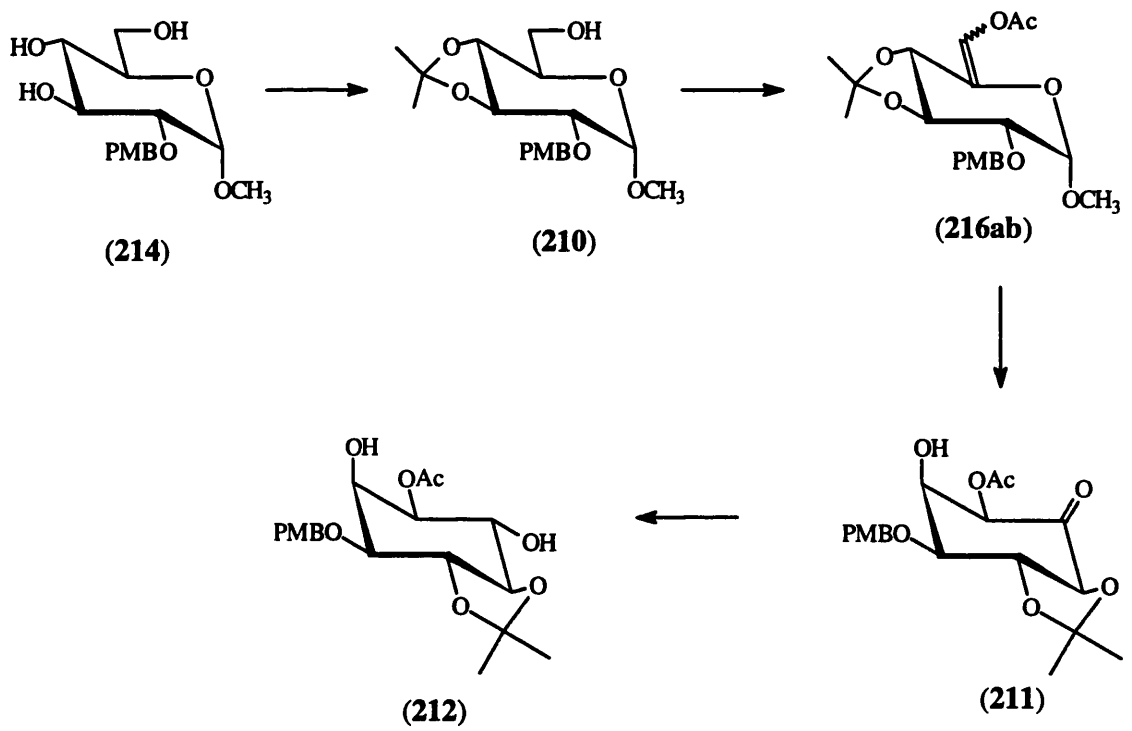


Fig. 4.3 Potential route to (**212**).

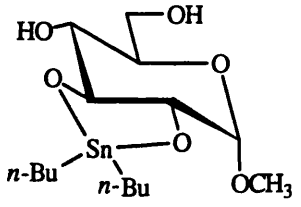
Thus, compound (**211**) was regarded as a desirable target and a potential synthetic route to it was investigated.

4.2 Discussion of Synthetic Work

4.2.1 Preparation of (*E,Z*)-Methyl 6-*O*-acetyl-3,4-*O*-isopropylidene-2-*O*-(*p*-methoxybenzyl)-α-D-*xylo*-hex-5-enopyranoside (216ab).

When methyl α-D-glucopyranoside (**142**) is reacted with one equivalent of dibutyltin oxide, the 2,3-*O*-dibutylstannylene (**213**) is preferentially formed.[218,262] Haque *et al.*[262] found that benzylation, allylation, methylation and methoxymethylation of (**213**) in either acetonitrile or dioxane gave 2-substituted ethers as major products in each case.

This author found that stannylation of (**142**) in methanol followed by *p*-methoxybenzylation in acetonitrile gave a mixture of at least six products by TLC. The required product (**214**) was isolated from the reaction mixture by a combination of column chromatography and fractional crystallisation. All attempts at obtaining pure (**214**) by crystallisation alone failed in the author's hands. Compound (**214**) was alternatively prepared by the hydrolysis of methyl 4,6-*O*-benzylidene-2-*O*-(*p*-methoxybenzyl)-α-D-glucopyranoside (**163**) (see chapter two) with aqueous acetic acid, thereby confirming its structure as the 2-substituted ether.



(**213**)

The use of isopropylidene ketals in carbohydrates is an important protection method which has been reviewed.[263,264] Where more than one monoketal product is possible in a molecule, *cis*-1,2-diols tend to be protected over 1,3-diols, which in turn tend to be favoured over *trans*-1,2-diols,[263,265] and these generalisations were confirmed in the present case. Thus, acid-catalysed reaction of (**214**) with 2-methoxypropene gave exclusively the 4,6-*O*-isopropylidene derivative (**215**). The required 3,4-*O*-isopropylidene isomer (**210**) was obtained in excellent yield by the sequential treatment

of (**214**) with *t*-butyldiphenylsilyl chloride,[266] 2-methoxypropene and tetrabutylammonium fluoride. The structures of (**210**) and (**215**) were distinguished between chiefly on the basis of their ^{13}C NMR spectra, which showed characteristic[267] chemical shift values for isopropylidene carbons in the proposed 1,3-dioxane (**215**) and 1,3-dioxolane (**210**) rings (fig. 4.4).

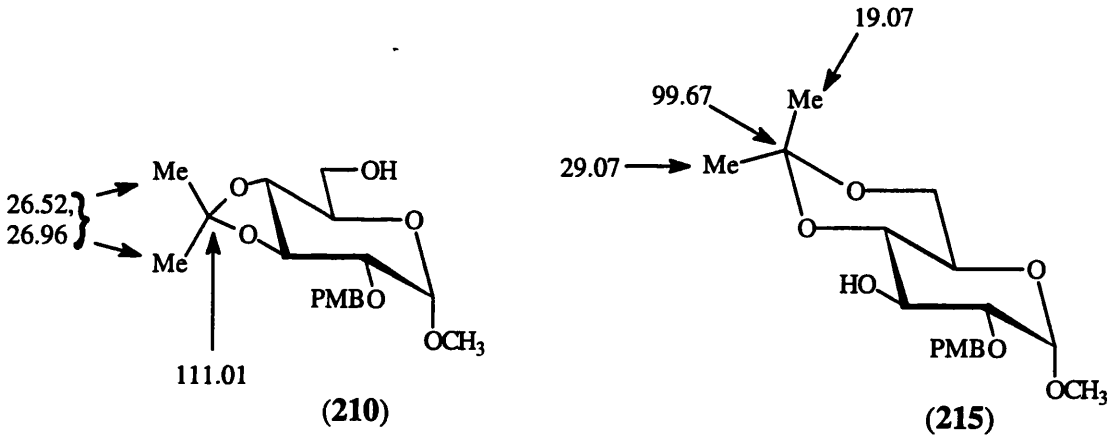
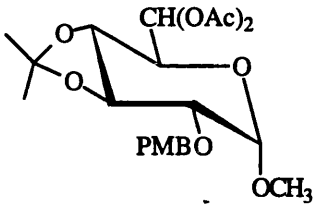


Fig. 4.4 ^{13}C NMR chemical shift values for isopropylidene carbons in (**210**) and (**215**).

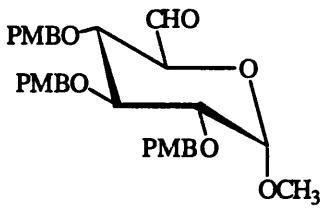
Enol acetate precursors to the modified Ferrier rearrangement are prepared by a two step process.[167] The primary alcohol is first oxidised to the corresponding aldehyde which is subsequently trapped in its enolate form by refluxing in a mixture of dry acetonitrile and acetic anhydride in the presence of potassium carbonate.

When (**210**) was subjected to the Swern oxidation followed by trapping of the crude product as above, only 26% of the required enol acetates (**216ab**) were isolated. The major product was the geminal diacetate (**217**) (43%), presumably formed by acetylation of the hydrated intermediate aldehyde. Estevez and Prestwich[191] reported that the intermediate aldehyde (**218**) was more than 90% hydrated. Partial purification of the aldehyde intermediate followed by azeotropic drying and trapping increased the yield of (**216ab**) to 70% with no concomitant production of (**217**). ^{1}H NMR spectroscopy indicated that (**216ab**) was formed as a mixture of geometrical isomers, in an approximate ratio of 4:1 by the integral ratio of the anomeric methyl signals. As the

stereochemical outcome of the Ferrier rearrangement is independent of the geometrical arrangement of the precursor enol acetate,[167] no attempt was made to separate these isomers. The major product was presumably the (*Z*)-isomer, in keeping with ample precedent.[167,191-193]



(217)



(218)

4.2.2 Attempted Ferrier Rearrangement of (216ab)

The modified Ferrier rearrangement involves treatment of a solution of the enol acetate in aqueous acetone with one equivalent of mercury (II) trifluoroacetate[167] or acetate[191] to open the carbohydrate ring, followed by sodium chloride to effect cyclohexanone ring closure.

When (**216ab**) was treated with mercury (II) acetate (chosen over the trifluoroacetate because of the acid lability of the isopropylidene ketal) as above, TLC indicated swift consumption of starting material to give a baseline product. Overnight stirring with enough sodium chloride to saturate the system caused consumption of the baseline product to give a less polar product. This was not the expected cyclohexanone (**211**), however, but 6-*O*-acetyl-3,4-*O*-isopropylidene-2-*O*-(*p*-methoxybenzyl)-D-*xylo*-5-hexosulose (**219**). The structure of (**219**) was assigned on the basis of its ^{1}H and ^{13}C NMR spectra. Part of the ^{1}H NMR spectrum of (**219**) and pertinent ^{13}C chemical shift values are shown in fig. 4.5.

Compound (**219**) corresponds to a hydroxymercurated ring-opened Ferrier intermediate (**220**; fig. 4.6) which, instead of ring closing, has lost mercury. Although Bender and Budhu[167] reported a resistance of the equivalent 2,3,4-tri-*O*-triethylsilyl analogue to ring closure, it was the organomercurial intermediate (**221**) which they isolated. In their case, ring closure was effected by treatment of (**221**) with various Lewis acids at –78°C,

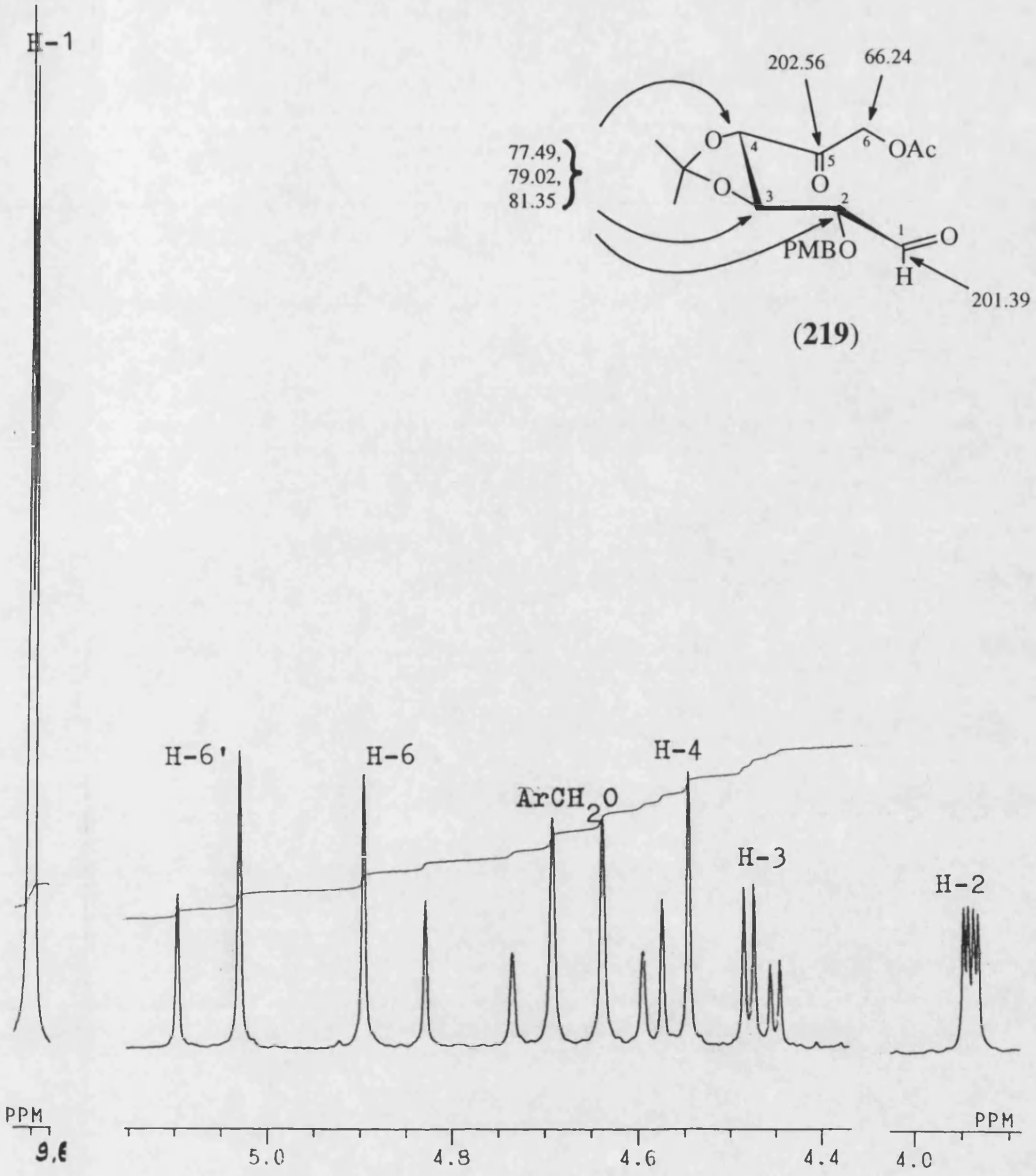
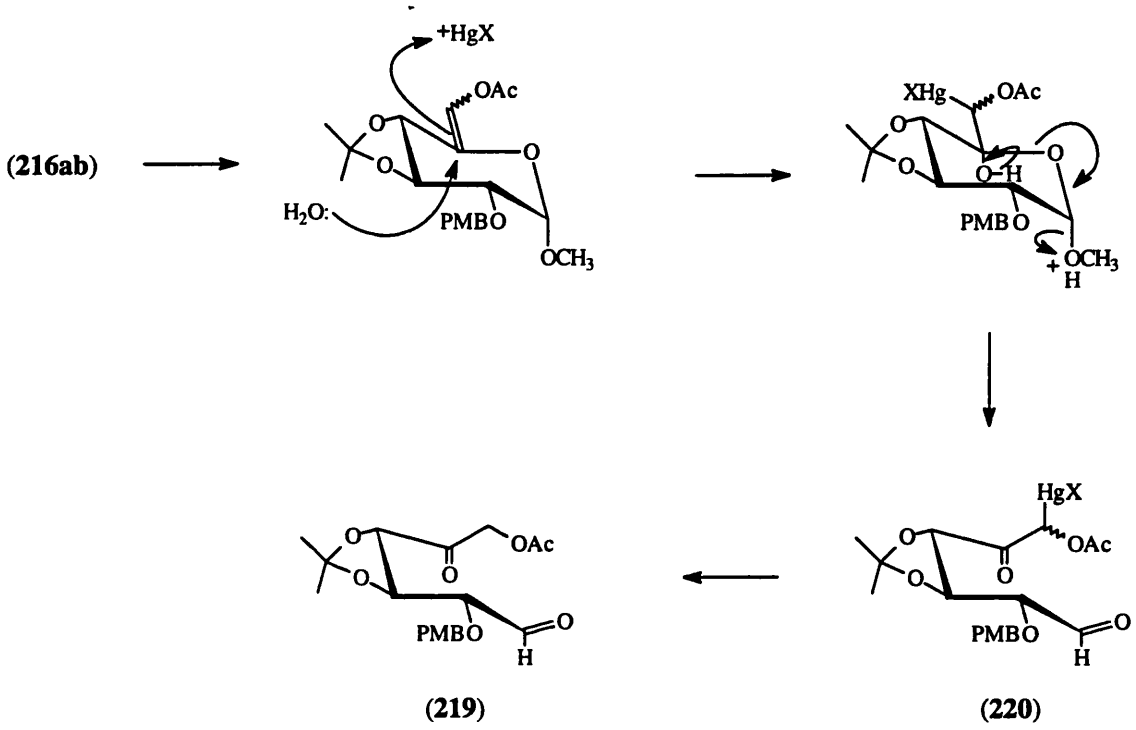


Fig. 4.5 Part of the 270MHz ^{1}H NMR spectrum of (**219**) in $CDCl_3$ and some ^{13}C NMR chemical shift values.

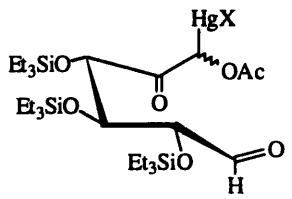
of which tin (IV) chloride was the best. The 6-deoxy-5-hexosulose product (**222**) was isolated by Blattner *et al.*[158] from a classical Ferrier rearrangement, and was rationalised as the result of acid-catalysed hydrolysis of the intermediate (**223**). When the product from (**216ab**) was worked up *immediately* after addition of sodium chloride, ^{1}H NMR analysis indicated the presence of a 1:1 diastereomeric mixture of organomercurial intermediates (**220**; duplicate H-6 singlets at 5.39 and 5.60ppm) in addition to (**219**).

The relative proportions of (**220**) (as mixture) and (**219**) was approximately 2:1 respectively, from the integral ratio of the H-6 singlets of (**220**) and the position 6 methylene AB quartet of (**219**). Addition of this mixture to a solution of tin (IV) chloride at –78°C gave an intractable mixture of at least four products by TLC, none of which contained the PMB group (*p*-methoxybenzylic compounds stain purple with PMA and heating).

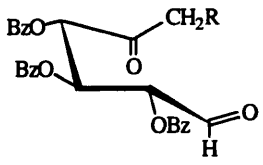


(**219**) (**220**)

Fig. 4.6 Possible mechanism for formation of (**219**) from (**216ab**).



(**221**)

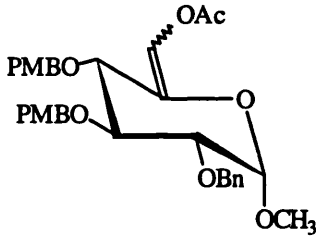


(**222**) R=H
(**223**) R=HgCl

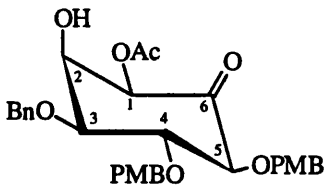
It is believed that the resistance of (**220**) to normal ring closure is due to the presence of the 3,4-*O*-isopropylidene protecting group, which is required to adopt a relatively strained *trans* formation for ring closure to occur. Although positions 3 and 4 of carbohydrate precursors have been incorporated into ring systems in other studies,[153,168] none has been a *trans*-fused five-membered ring.

4.2.3 Preparation of D-1-*O*-Acetyl-3-*O*-benzyl-4,5-di-*O*-(*p*-methoxybenzyl)-*myo*-inos-6-ose (225)

The failure of (**216ab**) to furnish the expected cyclohexanone coincided with the successful preparation of methyl 2-*O*-benzyl-3,4-di-*O*-(*p*-methoxybenzyl)-α-D-glucopyranoside (**185**), as described in chapter three. It was decided to continue the route with this compound for two reasons. First, because successful conversion of (**224ab**) to (**225**) would provide supporting evidence that the resistance of (**216ab**) to ring closure was attributable to the isopropylidene acetal and second, because although (**225**) was not as versatile an intermediate as (**211**), it did offer the prospect of furnishing some chiral analogues of Ins(1,4,5)P_3 modified at position 6.



(**224ab**)



(**225**)

Conversion of (**185**) to enol acetates (**224ab**) was achieved in 86% yield using the methodology described above. Treatment of a solution of (**224ab**) in aqueous acetone with mercury (II) trifluoroacetate followed by sodium chloride gave (**225**), obtained in 36% yield by a combination of column chromatography and crystallisation.

The structure of (**225**) was assigned on the basis of its ^1H and ^{13}C NMR spectra. Part of the ^1H 2D COSY spectrum of (**225**) is shown in fig. 4.7. All five ring methines have been assigned. In addition, the ^{13}C NMR spectrum exhibited signals from five inosose

ring methines and a quaternary carbon at 198ppm corresponding to C-6. Characteristic carbohydrate signals were absent.

With (**225**) in hand, attention turned to protection of the hydroxyl group at position 2. Attempted benzyloxymethylation with benzyloxymethyl chloride in dry acetonitrile containing proton sponge and quaternary ammonium salts,[191] or benzylation with benzyl bromide and silver (I) oxide in DMF gave mixtures of several *p*-methoxybenzyl-containing compounds by TLC, which presumably arose by β-elimination and subsequent aromatisation.

Lack of time prevented further exploration of this pathway.

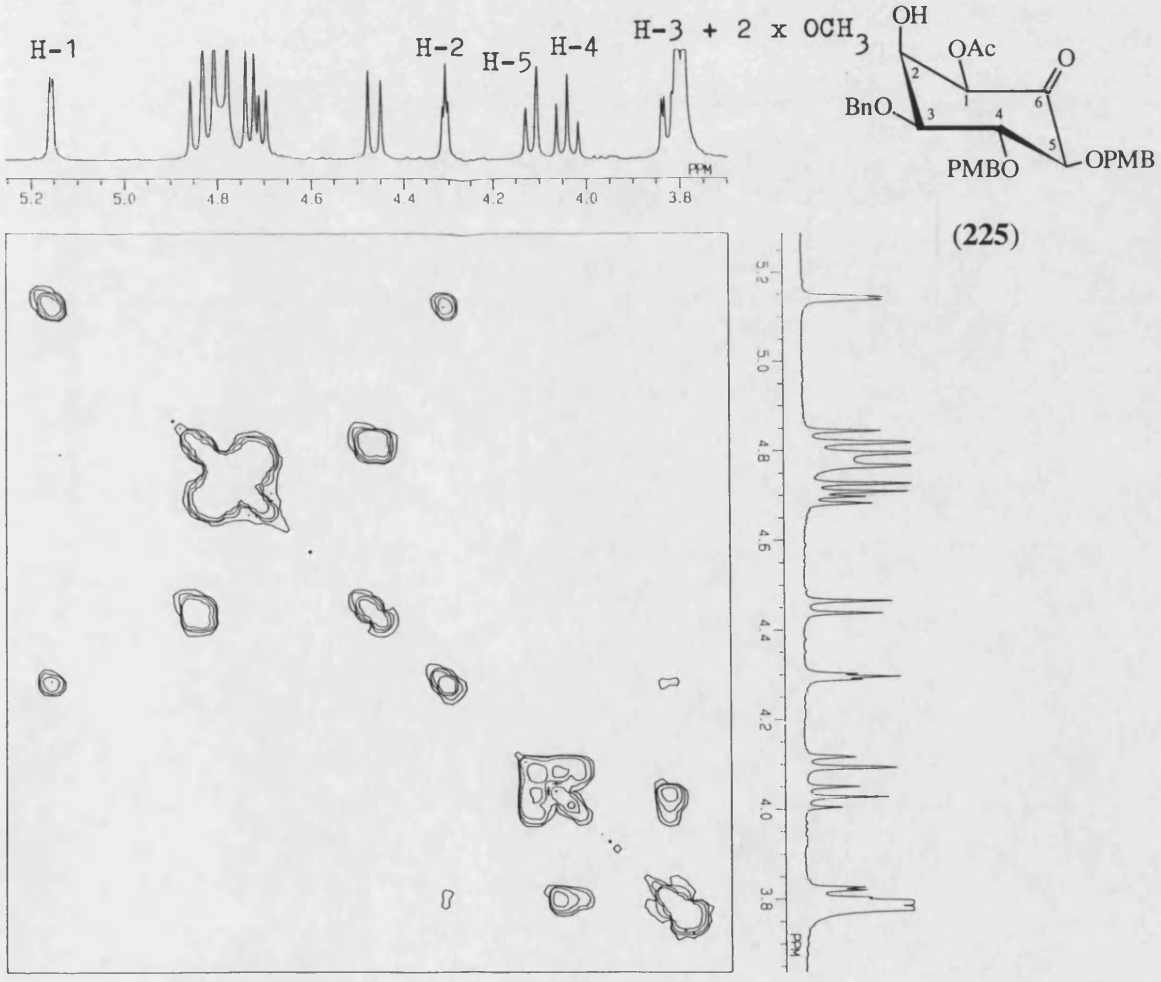
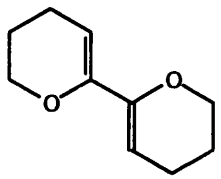


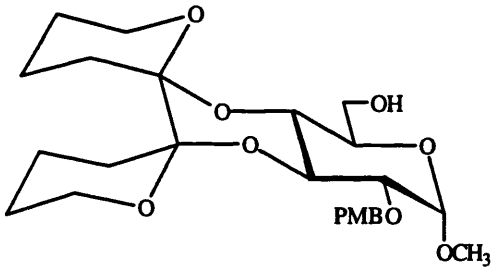
Fig. 4.7 Part of the 400MHz 2D ^{1}H COSY NMR spectrum of (**225**) in $CDCl_3$.

4.3 Suggestions for Further Work

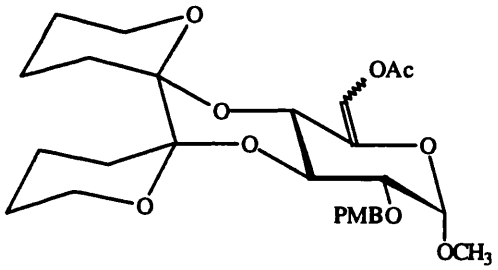
Ley and co-workers[268] have developed the "dispiroketal" protecting group for selective protection of *trans* vicinal diols. The use of this group is attractive in the above route for two reasons. First, because reaction of (**214**) with 3,3'4,4'-tetrahydro-6,6'-bi-2-*H*-pyran (**226**) would be expected to furnish the 3,4-*O*-acetal (**227**) directly, thus avoiding the need for protection and subsequent deprotection of the primary hydroxyl group. Second, the 3,4-diol of the enol acetates (**228ab**) would be contained within a six-membered ring, which, experiencing less strain than the isopropylidene, might be expected to allow the ring closure to cyclohexanone (**229**). Reduction of (**229**) should provide the *myo*-inositol derivative (**230**).



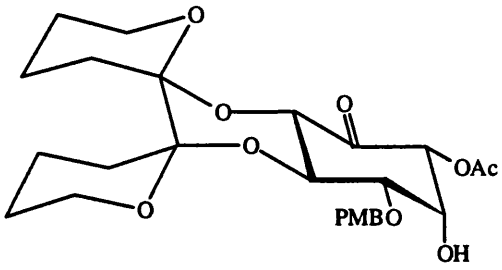
(**226**)



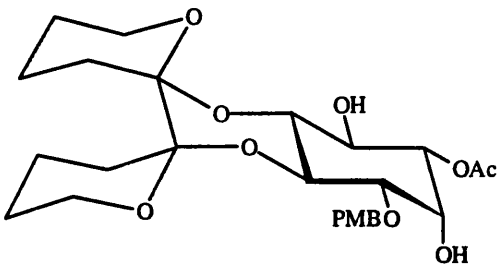
(**227**)



(**228ab**)



(**229**)



(**230**)

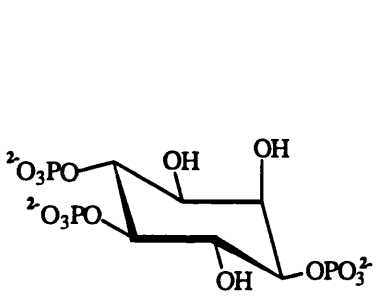
CHAPTER FIVE

SYNTHESIS OF INTERMEDIATES AND ANALOGUES OF Ins(1,4,5)P_3 BASED ON ADENOPHOSTIN A

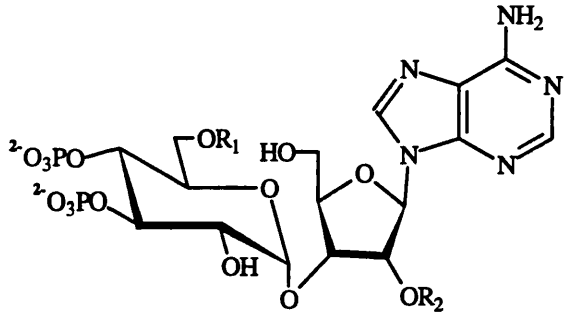
5.1 Introduction

5.1.1 General Introduction

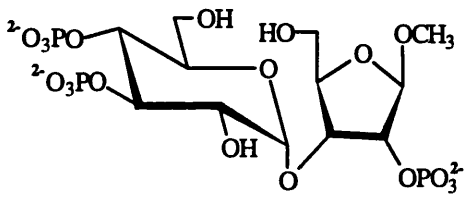
Although most of the active inositol polyphosphates and related compounds tested for Ca^{2+} release at the Ins(1,4,5)P_3 receptor during 1987–93 had been full agonists, most exhibited potency comparable to or less than that of the natural ligand (reviewed in chapter one). However, in late 1993 a Japanese group reported the isolation of two potently agonistic trisphosphates from a culture broth of *Penicillium brevicompactum*.[269] These were named adenophostins A and B, and identified as 3'-*O*-(α-D-glucopyranosyl)-adenosine-2',3",4"-trisphosphate (**231**) and its 6"-*O*-acetyl derivative (**232**) respectively.[270] The structure of (**231**) has recently been confirmed by total synthesis.[271]



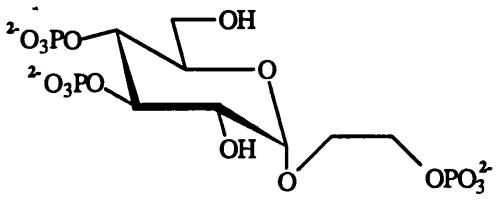
Ins(1,4,5)P_3



(**231**) R_1=H; R_2=PO_3^{2-}
(**232**) R_1=Ac; R_2=PO_3^{2-}
(**233**) R_1=R_2=H



(**234**)



(**235**)

The adenophostins have been demonstrated to be heparin-sensitive full agonists in rat cerebellar microsomes, with potencies 100-fold higher than Ins(1,4,5)P_3 [EC_{50} values

1.4nM (**231**) and 1.5nM (**232**); *cf.* 170nM Ins(1,4,5)P_3];[272] these relative potencies are consistent with binding data.[272] In another study involving the purified Ins(1,4,5)P_3 type I receptor, (**232**) was found to be 10-fold more potent than Ins(1,4,5)P_3 [EC_{50} values 11nM (**232**); *cf.* 100nM Ins(1,4,5)P_3].[273] In this study, (**232**) demonstrated a positive cooperativity in binding to the Ins(1,4,5)P_3 receptor not exhibited by Ins(1,4,5)P_3. Both (**231**) and (**232**) are resistant to the metabolic enzymes 5-phosphatase and 3-kinase and, as expected, produce a sustained Ca^{2+} release.[272]

5.1.2 Structure-Activity Considerations

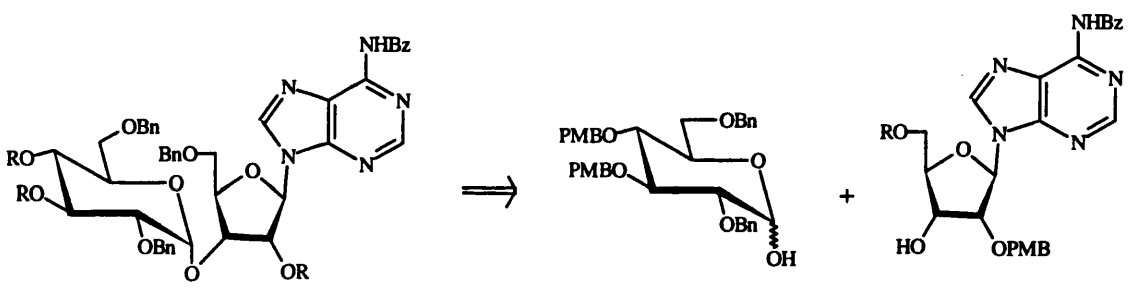
The high potency of the adenophostins is intriguing, as they bear little apparent resemblance to Ins(1,4,5)P_3. However, the structures of (**231**) and (**232**) show consistencies with many of the generalisations discussed in chapter one.

The glucose 3,4-bisphosphate moiety possesses D-*threo* stereochemistry and the position 2 hydroxyl group may be regarded as analogous to position 6. Indeed, molecular modelling studies[272,274] demonstrate similarity of positions 4, 3 and 2 of (**231**) with positions 4, 5 and 6 respectively of Ins(1,4,5)P_3. In addition, the adenophostins possess a third phosphate, which, similarly to Ins(1,4,5)P_3, is essential for high potency: 3'-*O*-(α-D-glucopyranosyl)-adenosine-3",4"-bisphosphate (**233**), in which this third phosphate is removed, possessed a 1000-fold lower binding affinity than (**231**).[272]

Although the broad basis for the activity of (**231**) and (**232**) is clear, a full structural rationalisation for their exceptional potency is lacking. An interesting combination of compounds to compare, for example, would be (**231**), methyl 3-*O*-(α-D-glucopyranosyl)-β-D-ribofuranoside-2,3',4'-trisphosphate (**234**) and (2-hydroxyethyl) α-D-glucopyranoside 2',3,4-trisphosphate (**235**). Such a study would establish the relative importance of the adenine and adenosine components of (**231**). The work described in this chapter represents initial attempts at preparing selectively protected glucose-based intermediates for synthesis of adenophostin A and related targets, and at preparing glucose-based polyphosphates.

5.1.3 Retrosynthetic Analysis

The preparation of adenophostin A would require deprotection of a fully protected, phosphorylated intermediate such as (**236**; fig. 5.1), derived from triol (**237**). Triol (**237**) could be obtained from a derivative in which positions 2', 3" and 4" are protected with a group removable in the presence of benzyl ethers, benzamides and glycosidic linkages. The *p*-methoxybenzyl ether fulfils this requirement. 6-*N*-Benzoyl-2'-*O*-(*p*-methoxybenzyl)-adenosine (**238**) is known,[275] and benzylation of the primary 5'-hydroxyl in favour of the secondary 3'-hydroxyl to give (**239**) ought to be straightforward. Selective coupling of (**239**) to position 1 of 2,6-di-*O*-benzyl-3,4-di-*O*-(*p*-methoxybenzyl)-D-glucopyranose (**240**) to provide the α-anomeric derivative (**241**) should be possible using trichloroacetimidate methodology commonly employed in oligosaccharide synthesis.[276]



(**236**) R=P(O)(OBn)$_2$
(**237**) R=H
(**241**) R=PMB

(**240**)

(**238**) R=H
(**239**) R=Bn

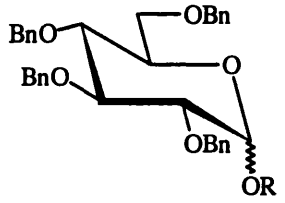
Fig. 5.1 Retrosynthetic analysis showing a potential route to (**231**).

The β-trichloroacetimidate derivative of (**240**) could also act as a glycosyl donor to other alcohols, such as 2-(*p*-methoxybenzyloxy)-ethanol, giving a precursor to (**235**), or methyl 5-*O*-benzyl-2-*O*-(*p*-methoxybenzyl)-β-D-ribofuranoside, giving a precursor to (**234**). Clearly, compound (**240**) is an important intermediate and, as part of a collaborative effort to synthesise adenophostin A analogues, the author sought a method to prepare (**240**).

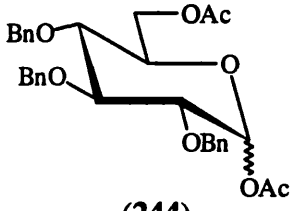
5.2 PREPARATION OF 2,6-DI-*O*-BENZYL-3,4-DI-*O*-(*p*-METHOXYBENZYL)-D-GLUCOPYRANOSE (240)

5.2.1 Attempted Preparation from the Methyl Glycoside

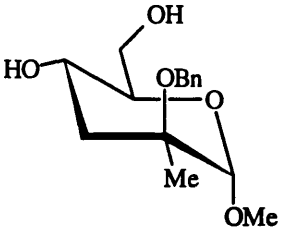
Two methods are commonly used to cleave methyl glycosides, namely acidic hydrolysis and acetolysis. Although 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose (**242**) has been prepared by refluxing a solution of the corresponding α-methyl glycoside (**243**) in *ca.* 2:1 acetic acid-1M sulphuric acid for 24 hours,[277] the acid-lability of *p*-methoxybenzyl ethers precludes this method as a route to (**240**).



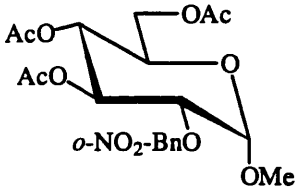
(**242**) R=H
(**243**) R= α- Me
(**253**) R=All



(**244**)



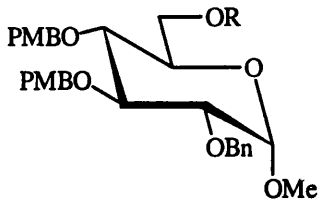
(**245**)



(**246**)

It is known that treatment of (**243**) with acetic anhydride containing 1% sulphuric acid rapidly produces 1,6-di-*O*-acetyl-2,3,4-tri-*O*-benzyl-D-glucopyranose (**244**),[278] *i.e.* cleavage of both the primary benzyl ether and the glycoside occurs. Leaving the reaction for longer (*ca.* 2 h) results in additional cleavage of the benzyl ethers at positions 2 and 3.[278] However, more recently, the milder catalyst boron trifluoride etherate has been used to acetolyse methyl 2-*O*-benzyl-3-deoxy-2-*C*-methyl-α-D-*arabino*-hexopyranoside (**245**)[279] and methyl 3,4,6-tri-*O*-acetyl-2-*O*-(*o*-nitrobenzyl)-α-D-glucopyranoside (**246**).[216] These reactions required treatment at 0°C for 4 h. Although

neither of these examples contained primary benzyl ethers, it was hoped that anomeric acetolysis would occur before benzyl ether cleavage.



(**247**) R=Bn
(**185**) R=H

Methyl 2,6-di-*O*-benzyl-3,4-di-*O*-(*p*-methoxybenzyl)-α-D-glucopyranoside (**247**) could easily be obtained by benzylation of (**185**), prepared in chapter three. Instead, an alternative route was explored. The synthesis of methyl 3-*O*-benzoyl-2-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (**144**; fig. 5.2), isolated without recourse to column chromatography on a 75g scale, was described in chapter two. Regioselective cleavage of the benzylidene acetal to give the primary benzyl ether (**248**), followed by saponification and *p*-methoxybenzylation would allow large amounts of (**247**) to be produced.

The required direction of benzylidene cleavage has been reported using sodium cyanoborohydride-hydrogen chloride,[280,281] aluminium chloride-triethylamine-borane complex[282] and trifluoroacetic acid-triethylsilane.[283] The first of these has been successfully applied to both methyl 2,3-di-*O*-benzyl- and 2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranosides,[280,281] and seemed ideally suited to (**144**).

Addition of dry hydrogen chloride in ether to a solution of (**144**) and sodium cyanoborohydride in THF at room temperature caused conversion of starting material to a single product. This was not characterised, but benzoylated, to provide methyl 3,4-di-*O*-benzoyl-2,6-di-*O*-benzyl-α-D-glucopyranoside (**249**), identifiable by deshielded triplets at 5.49 and 5.92ppm in its ^{1}H NMR spectrum, corresponding to H-3 and H-4 respectively, and thereby confirming the direction of benzylidene cleavage. Compound (**249**) was used as a model for the acetolysis reaction.

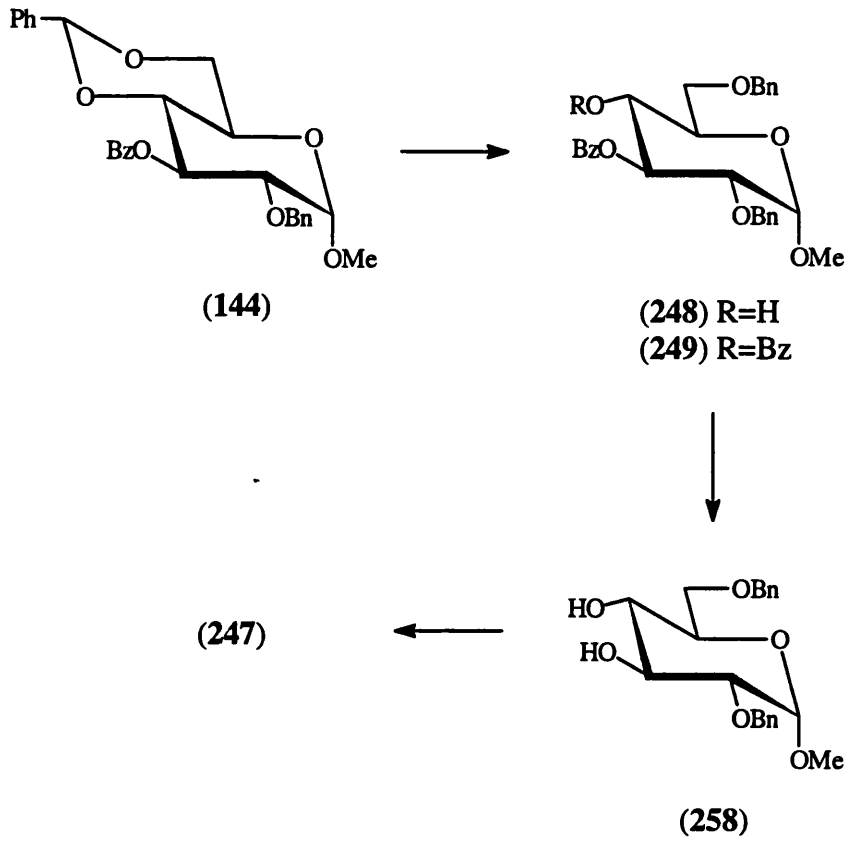


Fig. 5.2 Potential route to (**247**) from (**144**).

Treatment of (**249**) with acetic anhydride and boron trifluoride etherate* at 0°C resulted in swift conversion of starting material to methyl 6-*O*-acetyl-3,4-di-*O*-benzoyl-2-*O*-benzyl-α-D-glucopyranoside (**250**; fig. 5.3; 64%) and 1,6-di-*O*-acetyl-3,4-di-*O*-benzoyl-2-*O*-benzyl-D-glucopyranose (**251**; 7%). In the ^{1}H NMR spectra of both (**250**) and (**251**), the deshielded two-proton signals demonstrated that the primary benzyl ether, and not the secondary, had been replaced by an acetate ester. Thus, primary ether cleavage is more rapid than deglycosylation. Presumably a small amount of methoxide loss occurs *via* the oxonium ion (**252**; fig. 5.3) initially. Methoxide then reacts with acetic anhydride to form methyl acetate and acetate anion, the nucleophile, which either recombines with the oxonium ion (route A) or cleaves the primary benzyl ether of another molecule of (**249**) (route B). This latter route generates benzyl acetate and alkoxide and a self-perpetuating pathway Attack of acetate at the benzyl ether methylene carbon is more likely than at C-6 because acetolysis of secondary benzyl

* Failure to dry this reagent freshly resulted in complete absence of reaction.

ethers proceeds with retention of configuration.[278] To the best of the author's knowledge, this is the first example of a selective benzyl ether acetolysis in the presence of a glycoside.

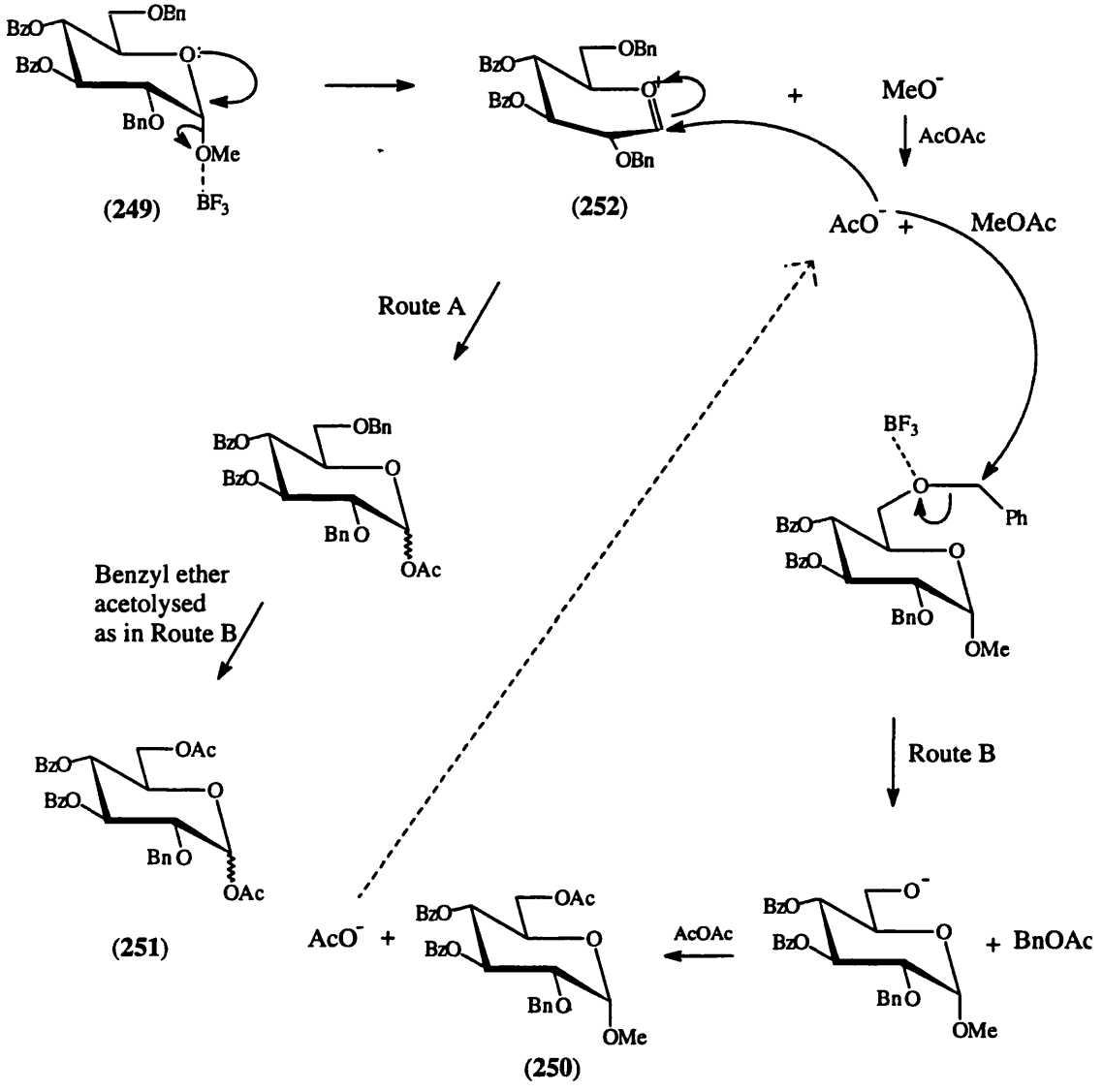


Fig. 5.3 Possible mechanism of formation of (**250**) and (**251**) from (**249**).

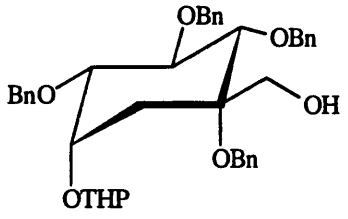
In conclusion, a route to (**240**) from the methyl glycoside was not convenient, and an alternative method was sought.

5.2.2 Preparation from the Allyl Glycoside

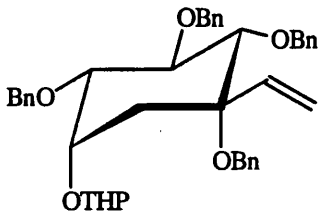
An alternative preparation of 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose (**242**) from the corresponding allyl glycosides has been described by Gigg and Gigg.[284] Allyl ethers have been widely used as protecting groups in carbohydrate and inositol chemistry because, while relatively stable themselves, they can be induced to isomerise to prop-1-enyl ethers by a variety of reagents.[285,286] These enol ethers are hydrolysed by mildly acidic (and other[285,286]) conditions.

Thus Gigg and Gigg prepared an anomeric mixture of allyl 2,3,4,6-tetra-*O*-benzyl-D-glucopyranosides (**253**), isomerised the allyl groups using potassium *t*-butoxide in dry DMSO at 100°C for 15 min and obtained (**242**) by refluxing a solution of the products in 9:1 acetone-0.1M aqueous HCl for 30 min.[284] Using essentially this methodology, allyl ethers have been removed in the presence of *p*-methoxybenzyl ethers,[100,287] making this an ideal route for the preparation of (**240**).

Selective protection of positions 2 and 6 of the allyl glycoside(s) is required in this route. As position 2 bears the most reactive secondary hydroxyl group in α-alkyl glycosides, whereas position 3 bears the most reactive in the β-isomers,[215,220,221] the preparation of allyl α-D-glucopyranoside (**254a**) was required. In addition, use of the α-allyl glycoside potentially allowed preparation of (2-hydroxyethyl) α-D-glucopyranoside 2',3,4-trisphosphate (**235**) directly: Ferrier and Stütz[185] had prepared (**255**) from (**256**) by reaction of the latter with osmium tetraoxide-sodium metaperiodate followed by reduction of the intermediate aldehyde with sodium borohydride. It followed that an allyl glycoside ought to be similarly convertible to a (2-hydroxyethyl) glycoside.

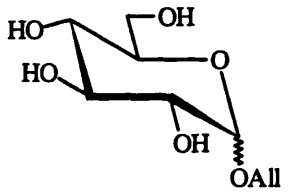


(**255**)



(**256**)

Glycoside (**254a**) was first obtained pure by reaction of D-glucose with dry allyl alcohol containing dissolved hydrogen chloride gas (*i.e.* a classical Fischer glycosylation), followed by dry acetone extraction and fractional crystallisation,[288] although a recent report[289] found this general method unsatisfactory. A more commonly used method is a modified Fischer glycosylation in which a mixture of D-glucose, allyl alcohol and a strong ion-exchange resin are heated under reflux.[289–291] When this author applied the latter procedure, an orange syrup was obtained after cooling, filtration and evaporation. In agreement with Lee and Lee,[290] no crystallisation could be induced at this stage. Column chromatography of this syrup gave a white solid (**254ab**). ^{1}H NMR spectroscopy of (**254ab**) in D_2O revealed a *ca.* 7:3 α:β anomeric mixture of allyl glycosides, estimated from the integral ratio of the anomeric protons, resonating at 4.92ppm (*J* 3.7Hz) and 4.46ppm (*J* 7.9Hz) respectively. A single crystallisation from ethyl acetate-ethanol typically gave a 9:1 α:β mixture. Fractional crystallisation gave the pure α-anomer (**254a**), but only in poor yield (14%).



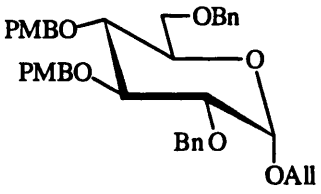
(**254ab**) 7:3 α:β
(**254a**) α-anomer

Attention now turned to selective protection of positions 2 and 6. In principle, allyl 2,6-di-*O*-benzyl-3,4-di-*O*-(*p*-methoxybenzyl)-α-D-glucopyranoside (**257**) could be prepared from the 4,6-*O*-(*p*-methoxybenzylidene) acetal using similar methodology to that described in chapter three, or from the 4,6-*O*-benzylidene acetal, as described for the methyl glycoside above. However, a more challenging and potentially more convenient approach would be to protect positions 2 and 6 simultaneously.

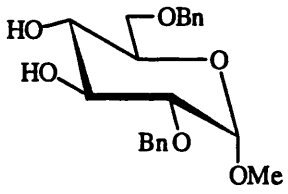
Methyl 2,6-di-*O*-benzyl-α-D-glucopyranoside (**258**) was produced in 30% yield when methyl α-D-glucopyranoside was treated with 1.5 equiv. of bis(tributyltin) oxide, followed by neat benzyl bromide,[255] presumably by formation of the 2,6-

bis(tributylstannyl) diether. A possible method of disubstitution of (**254a**) would therefore be to react it with 2 equiv. of bis(tributyltin) oxide and to benzylate it as above. An alternative possibility was to extend the use of stannylene acetals.

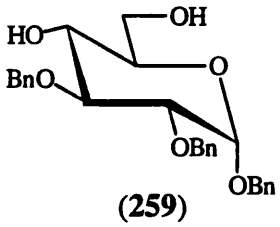
Reaction of methyl α-D-glucopyranoside with 1 equiv. of dibutyltin oxide results in formation of the 2,3-*O*-dibutylstannylene derivative, with selective esterification[218] or alkylation[262] occurring at position 2. In addition, benzyl 2,3-di-*O*-benzyl-α-D-glucopyranoside (**259**) has been regioselectively methoxymethylated at position 6 *via* its 4,6-*O*-dibutylstannylene derivative.[292] Therefore, it seemed reasonable that reacting an alkyl glucopyranoside with more than 2 equiv. of dibutyltin oxide ought to result first in the formation of the 2,3-*O*-dibutylstannylene, followed by the 2,3:4,6-di-*O*-dibutylstannylene. The latter derivative of an α-anomer (**260**) would be expected to direct substitution at positions 2 and 6.



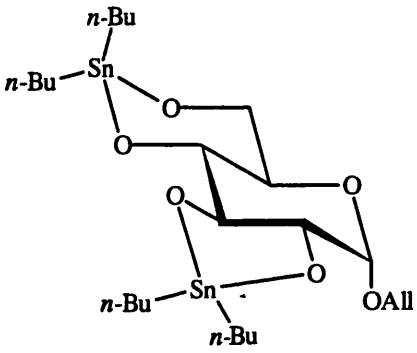
(**257**)



(**258**)



(**259**)



(**260**)

As the α-anomer (**254a**) had been more difficult than expected to isolate from the glycosylation reaction, an ideal situation would be the isolation of a 2,6-disubstituted α-anomeric derivative directly from (**254ab**). Such methodology would be potentially of

general use to carbohydrate chemists and consequently, it was decided to attempt both esterification and alkylation using a bis stannylene approach.

When (**254ab**) was reacted with 2.5 equiv. of dibutyltin oxide in toluene and the reaction mixture cooled, a precipitate formed which could not be redissolved in toluene or dioxane. Hoping that gradual dissolution and reaction would occur, this suspension (in toluene) was stirred for several days with 2.1 equiv. of benzoyl chloride, but no reaction occurred. After completion of these studies, Qin and Grindley[293] noted the poor solubility of methyl 2,3:4,6-di-*O*-dibutylstannylene-α-D-glucopyranoside in chloroform and suggested oligomerisation.

The stannylation product of (**254ab**) with 1.05–1.2 equiv. of dibutyltin oxide did not precipitate on cooling. Treatment of the cooled solution with 2.1 equiv. of benzoyl chloride gave a mixture of products by TLC, from which the known[291] allyl 2,6-di-*O*-benzoyl-α-D-glucopyranoside (**261**; fig. 5.4) was isolated in 34% yield by a combination of column chromatography and crystallisation. Presumably the 2,3-*O*-dibutylstannylene derivative of the α-anomer formed, was soluble in cold toluene and directed substitution at position 2, while the primary hydroxyl was selectively benzoylated over the remaining free secondary hydroxyl at position 4 as expected. This two-step preparation of (**261**) from D-glucose represents an improvement on the five steps of Pelyvás *et al.*[291]

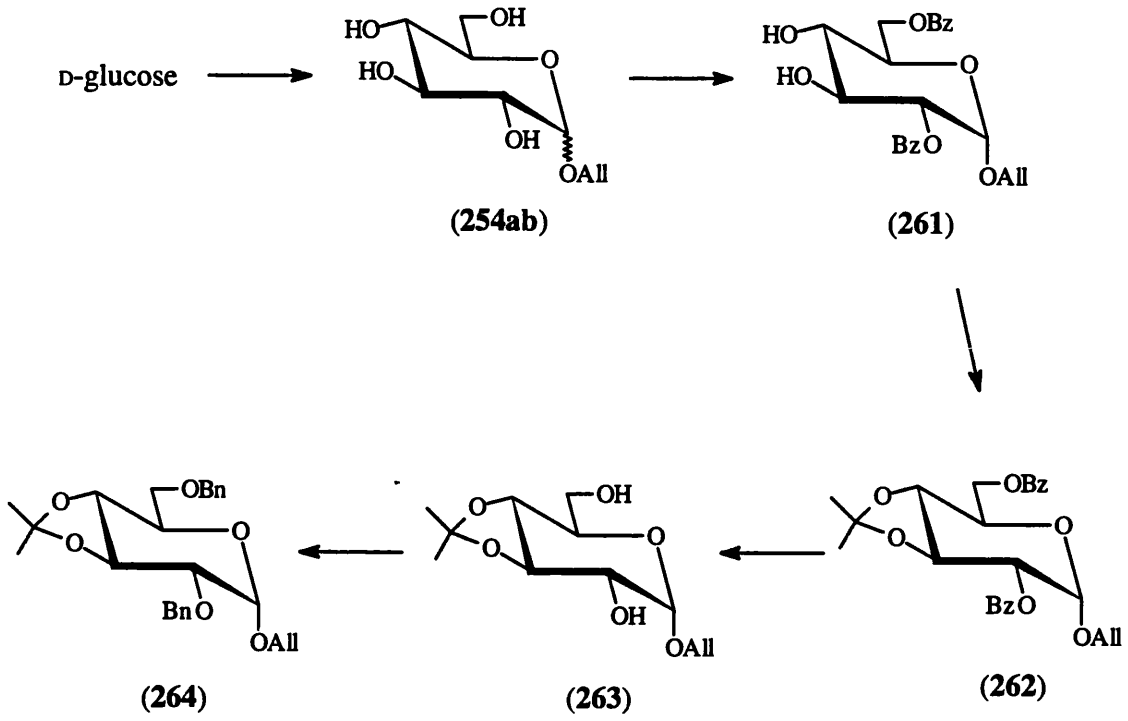
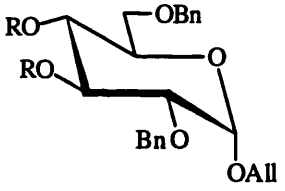


Fig. 5.4 Route to (**264**) from D-glucose *via* dibenzoate (**261**).

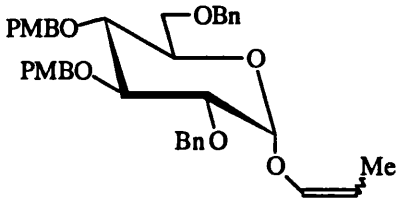
Reaction of (**261**) with 2-methoxypropene gave fully protected (**262**; fig. 5.4). It is noteworthy that best yields of 3,4-*O*-isopropylidene derivatives were obtained with a short reaction time. To the best of the author's knowledge, the publication of this procedure[294] represented the first literature example of the use of an isopropylidene acetal to protect positions 3 and 4 of a glucose derivative. Recently, a variation of this method has been described on the enantiomer of (**261**) using THF as solvent over a longer period.[295] The benzoate esters of (**262**) were easily replaced with benzyl ethers in two-steps: basic methanolysis provided diol (**263**) which was benzylated with sodium hydride and benzyl bromide in DMF to provide allyl 2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-α-D-glucopyranoside (**264**).

Attention now turned to potential direct benzylation. Alkylation is carried out at higher temperatures than esterification, at which the bis stannylene intermediate (**260**) might be soluble. As benzylation tends to be less selective than benzoylation, the pure α-anomer (**254a**) was used initially to test the viability of this method.

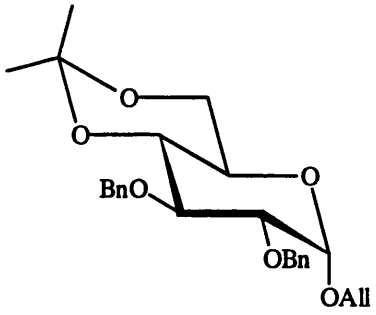
Treatment of the stannylated product with benzyl bromide in refluxing toluene or acetonitrile (both containing quaternary ammonium salts) resulted in sluggish reactions providing many products by TLC, none of which dominated and which were not completely separable by column chromatography. However, when stirred with neat benzyl bromide at 100–110°C, the stannylated product dissolved within one hour and after two days a major product and several minor products (of similar R_f value) were obtained. After work up and column chromatography to remove excess benzyl bromide, attempts were made to crystallise the major product. Although no crystallisation could be induced with most common solvents (methanol, ethanol, isopropanol, diethyl ether, ethyl acetate-hexane), diisopropyl ether yielded a single dibenzyl derivative in 44% yield (two crops). This was identified as the required allyl 2,6-di-*O*-benzyl-α-D-glucopyranoside (**265**) by preparation of its 3,4-dibenzoate (**266**), the ^{1}H NMR spectrum of which displayed deshielded triplets at 5.52 and 5.98ppm corresponding to H-4 and H-3 respectively, and by reaction with 2-methoxypropene to provide (**264**).



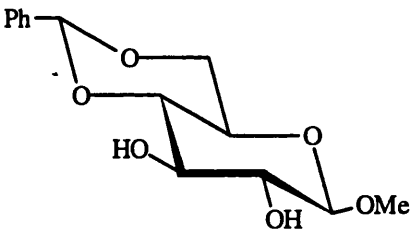
(**257**) R=PMB
(**265**) R=H
(**266**) R=Bz



(**269ab**)



(**267**)



(**268**)

In the hope that further quantities of (**265**) could be obtained *via* the less polar (**264**), the mother liquor of the benzylation mixture was reacted with 2-methoxypropene. A single new product was formed, which was easily isolated by flash chromatography. This

compound was not (**264**), however, but a dibenzyl derivative possessing an isopropylidene acetal in a 1,3-dioxane chair conformation, as apparent from the characteristic[267] chemical shifts of the isopropylidene axial methyl (19.18ppm), equatorial methyl (29.22ppm) and quaternary (99.34ppm) carbons in the ^{13}C NMR spectrum in $CDCl_3$. Its structure was therefore assigned as allyl 2,3-di-*O*-benzyl-4,6-*O*-isopropylidene-α-D-glucopyranoside (**267**), thereby demonstrating that all of (**265**) had been isolated by crystallisation.

With appropriate benzylation methodology and a convenient isolation procedure established, the technique was attempted on (**254ab**) on a 35g scale. In this case (**265**) could still be crystallised from the (more complicated) product mixture, but only in poor yield (*ca.* 15%). Reaction of the mother liquor with 2-methoxypropene in this case gave a mixture of several acetal-containing species which could not be separated further. Although the yield of crystalline (**265**) from the anomeric mixture was rather disappointing, this method has been found to be the most convenient overall to produce it in multigram quantities.

While this work was in progress, Qin and Grindley[293] reported similar benzylation selectivity on methyl α-D-glucopyranoside, confirming the general applicability of this method. In their studies, conducted on a 1mmol scale, only the 2,6-dibenzyl derivative was obtained, and an improved yield was reported when using only 1.5 equiv. of dibutyltin oxide. This has not been attempted on (**254a**). Another interesting result was the product mixture obtained from methyl β-D-glucopyranoside: the 2,6-di-*O*-benzyl derivative was the major product (56%) and not the 3,6-di-*O*-benzyl derivative (16%). This selectivity is the reverse of that observed with the 4,6-*O*-benzylidene derivative (**268**) which gave a *ca.* 1:2 ratio of the 2- and 3-*O*-benzyl ethers,[296] although it is consistent with the ratio of monoalkylated products from use of 1 equiv. of dibutyltin oxide with methyl β-D-glucopyranoside.[262]

The conversion of (**265**) to target (**240**) was achieved in three steps. *p*-Methoxybenzylation of (**265**) with sodium hydride and *p*-methoxybenzyl chloride gave fully protected (**257**), which was isomerised to the corresponding prop-1-enyl glycosides (**269ab**) using potassium *t*-butoxide in dry DMSO. Finally, hydrolysis with refluxing

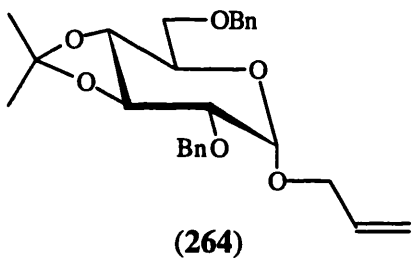
10:1 acetone-1M aqueous HCl for 20 min gave (**240**) in 77% yield after crystallisation. Compound (**240**) was identified by positive Fehling's test and by its ^{1}H NMR spectrum, which indicated its solution in $CDCl_3$ to be a *ca.* 1:1 anomeric mixture.

Use of (**240**) in trichloroacetimidate coupling methodology is currently being investigated in these laboratories.

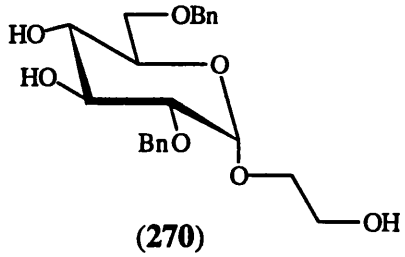
5.3 Preparation of (2-Hydroxyethyl) α-D-glucopyranoside 2',3,4-trisphosphate (235)

Attention now turned to direct preparation of (**235**), which represents the derivative of adenophostin A in which most of the adenosine moiety has been deleted.

As both benzyl ethers[185] and isopropylidene acetals[297] should be stable to osmium tetraoxide, (**264**) was chosen as an appropriate selectively protected intermediate for the synthesis of (**235**). Reaction of (**264**) with 0.3 equiv. of osmium tetraoxide and excess sodium metaperiodate for 5 h produced a polar product which was not isolated but was reduced with sodium borohydride to furnish (2-hydroxyethyl) 2,6-di-*O*-benzyl-α-D-glucopyranoside (**270**) in 56% overall yield from (**264**). The structure of (**270**) was assigned chiefly on the basis of its ^{13}C NMR spectrum (fig. 5.5) which showed loss of isopropylidene and allyl signals and the presence of methylene carbons at 61.47 and 70.28ppm, typical[298] of the 2' and 1' positions respectively of an α-(2-hydroxyethyl) glucoside.

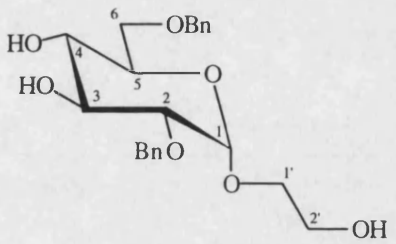


(**264**)



(**270**)

Loss of the isopropylidene acetal in this reaction was unexpected. It could not have occurred in the presence of sodium metaperiodate, as the resulting diol would have been oxidised to a dialdehyde. Noting the higher solubility of osmium tetraoxide in organic solvents compared to water,[299] it presumably partitioned into the organic layer during purification of the intermediate aldehyde and was subsequently reduced to osmic acid (H_2OsO_4), an acid sufficiently strong to remove the labile *trans* ketal. Nevertheless, the loss of the acetal was advantageous, as it directly provided the triol required for phosphorylation.



(**270**)

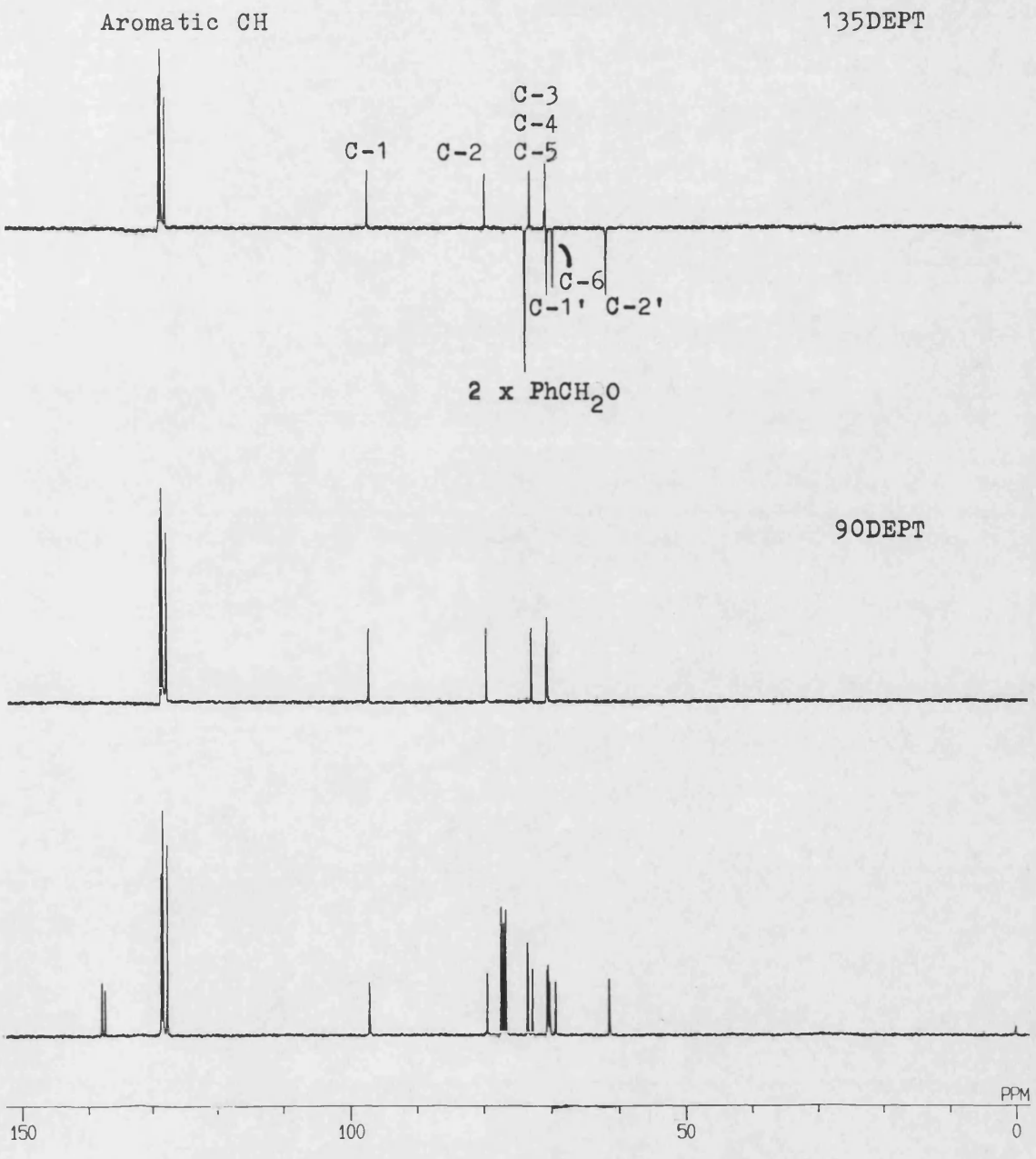


Fig. 5.5 100MHz ^{13}C NMR spectrum of (**270**) in $CDCl_3$.

Phosphitylation of (**270**) with tetrazole-activated bis(benzyloxy)(diisopropylamino)-phosphine gave a trisphosphite triester, in which the vicinal bisphosphite presented as an AB quartet and the primary phosphite as a singlet in the ^{31}P NMR spectrum (fig. 5.6). After oxidation with MCPBA and purification, the ^{1}H-coupled ^{31}P NMR spectrum of the product (**271**; fig. 5.7) exhibited a septet corresponding to the phosphorylated primary alcohol and two sextets corresponding to the protected ring phosphates.

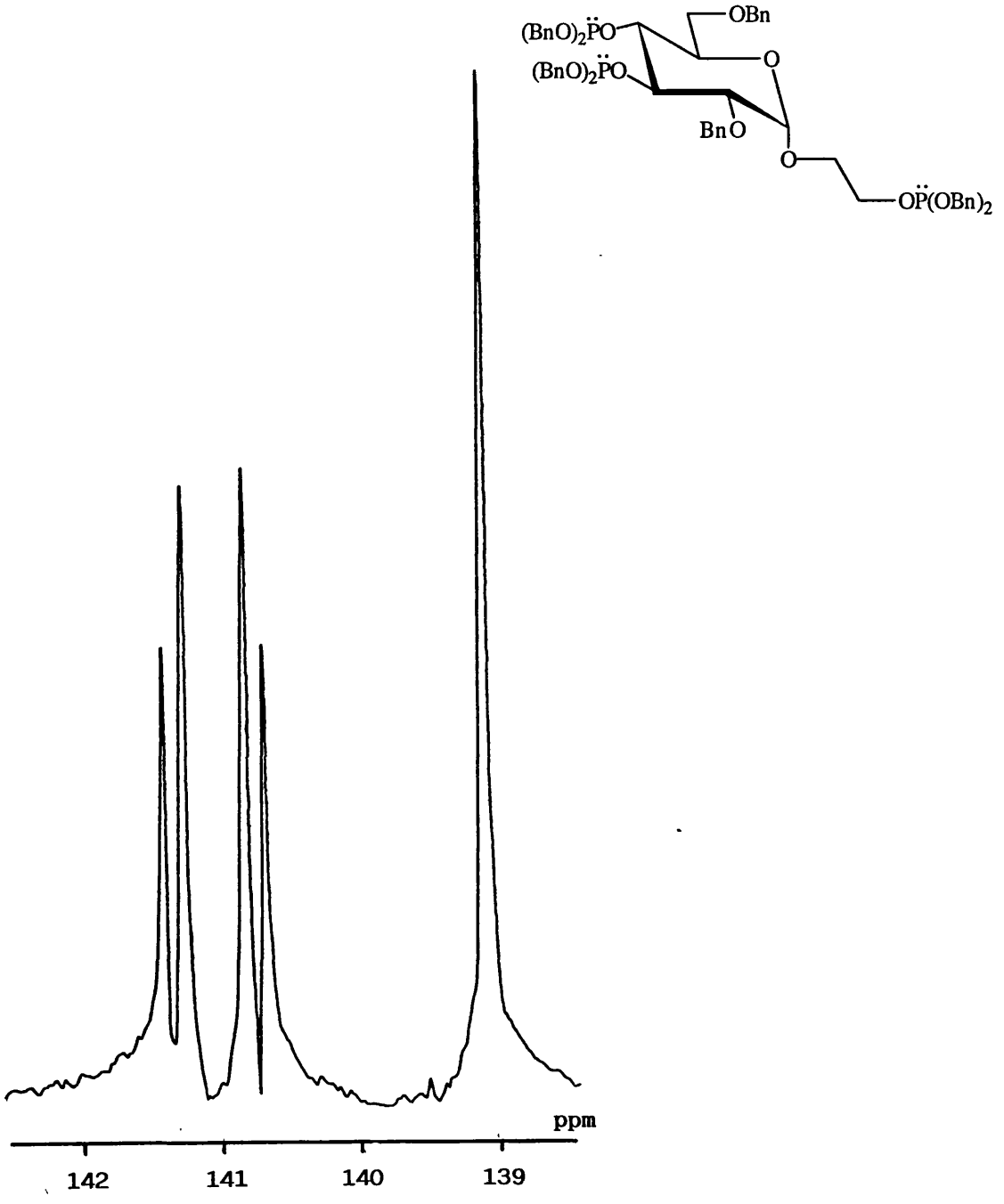
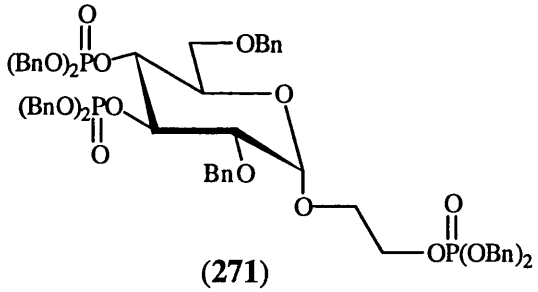


Fig. 5.6 36MHz ^{31}P NMR spectrum of trisphosphite triester of (**270**) in CH_2Cl_2.



(271)

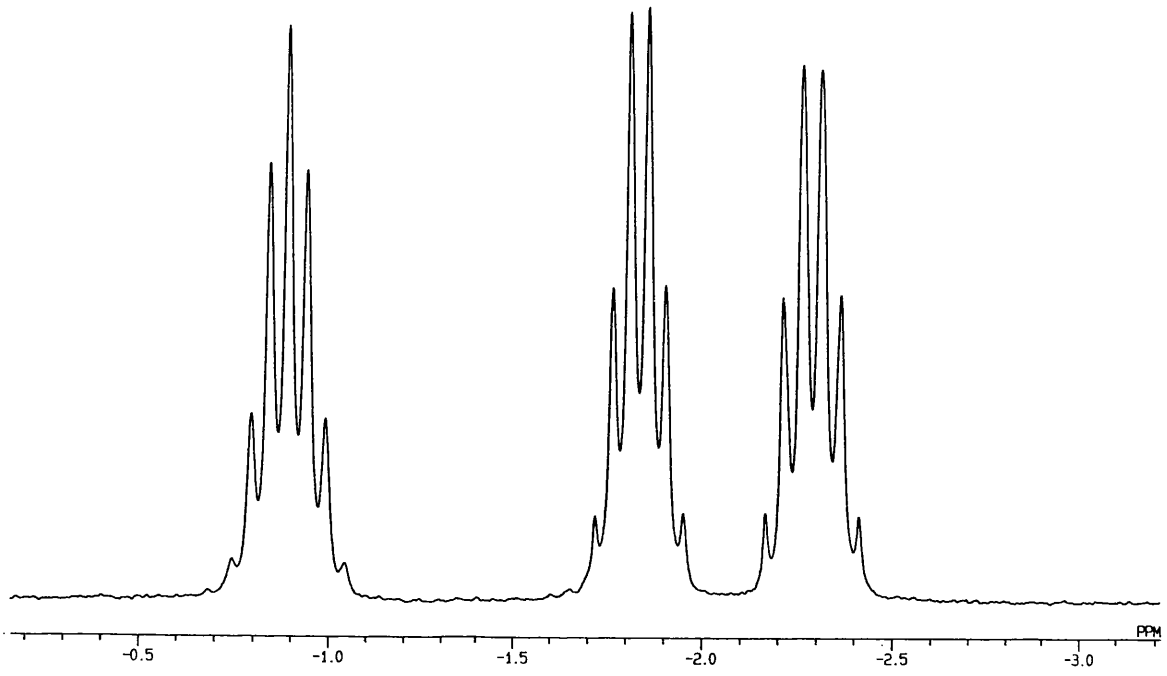


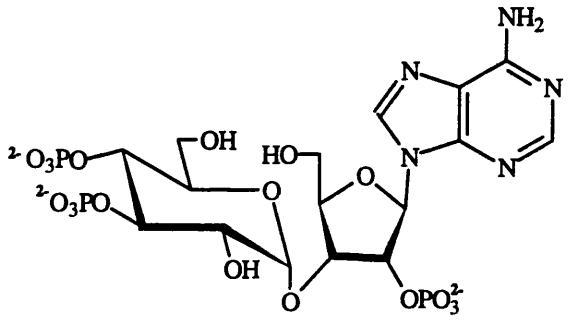
Fig. 5.7 162MHz ^{1}H-coupled ^{31}P NMR spectrum of (**271**) in $CDCl_3$.

Deprotection of (**271**) with sodium in liquid ammonia, followed by purification by ion-exchange chromatography, provided the required trisphosphate (**235**), isolated as the triethylammonium salt and quantified by Briggs phosphate assay.

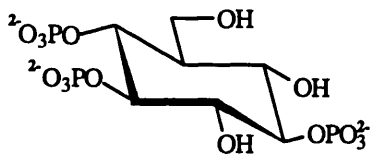
Trisphosphate (**235**) has also been prepared by Gigg and co-workers from D-galactose;[300] to date only its biological evaluation has been published.[274]

5.4 Biological Data for (2-Hydroxyethyl) α-D-glucopyranoside 2',3,4-trisphosphate

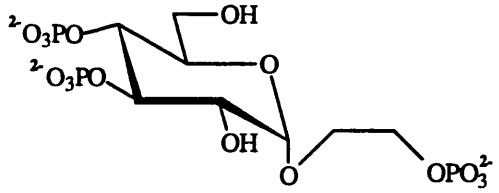
Trisphosphate (**235**) was examined for Ca^{2+}-mobilising activity at the platelet Ins(1,4,5)P_3 receptor. It was found to be a full agonist with a potency *ca.* 10-fold lower than that of Ins(1,4,5)P_3 [EC_{50} 0.6μM; *cf.* Ins(1,4,5)P_3 0.05μM, fig. 5.8], a result consistent with binding data (fig. 5.9) and with another study in SHSY5Y neuroblastoma cells.[274]



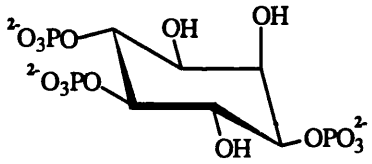
Adenophostin A (**231**)



(**272**)



(**235**)



Ins(1,4,5)P_3

Two related studies are of interest to this discussion. First, DL-3-deoxy-3-hydroxymethyl-*scyllo*-inositol-1,2,5-trisphosphate (**272**) demonstrated a Ca^{2+}-mobilising potency approximately equal to that of Ins(1,4,5)P_3 at the platelet Ins(1,4,5)P_3 receptor [EC_{50} 0.1μM; *cf.* Ins(1,4,5)P_3 0.11μM].[301] Assuming the L-enantiomer is inactive, and noting that D-*scyllo*-Ins(1,2,4)P_3 has approximately equal Ca^{2+}-mobilising potency to

$Ins(1,4,5)P_3$, this implies that the CH_2OH component is at least tolerated by the $Ins(1,4,5)P_3$ receptor, and may even give rise to a modest increase in potency. The observation that (**235**) is less active than either (**272**) or $Ins(1,4,5)P_3$ suggests that the conformationally rather mobile 2'-phosphate group of (**235**) is not a good mimic of the 2'-phosphate in adenophostin A, nor of the 1-phosphate of $Ins(1,4,5)P_3$. Thus, all or part of the adenosine moiety in adenophostin A may be necessary to orientate the 2'-phosphate group in a particularly favourable way at the receptor binding site. The biological data above clearly demonstrate that at least part of the adenosine component of the adenophostins is essential for the extreme potency exhibited by these glyconucleotides. Furthermore, (**235**) represents the first carbohydrate-based trisphosphate to exhibit any significant agonistic activity at the $Ins(1,4,5)P_3$ receptor. It therefore complements and consolidates the other work in this thesis, showing that structurally diverse polyphosphates which retain the pharmacophore defined in chapter one are able to mobilise intracellular Ca^{2+}.

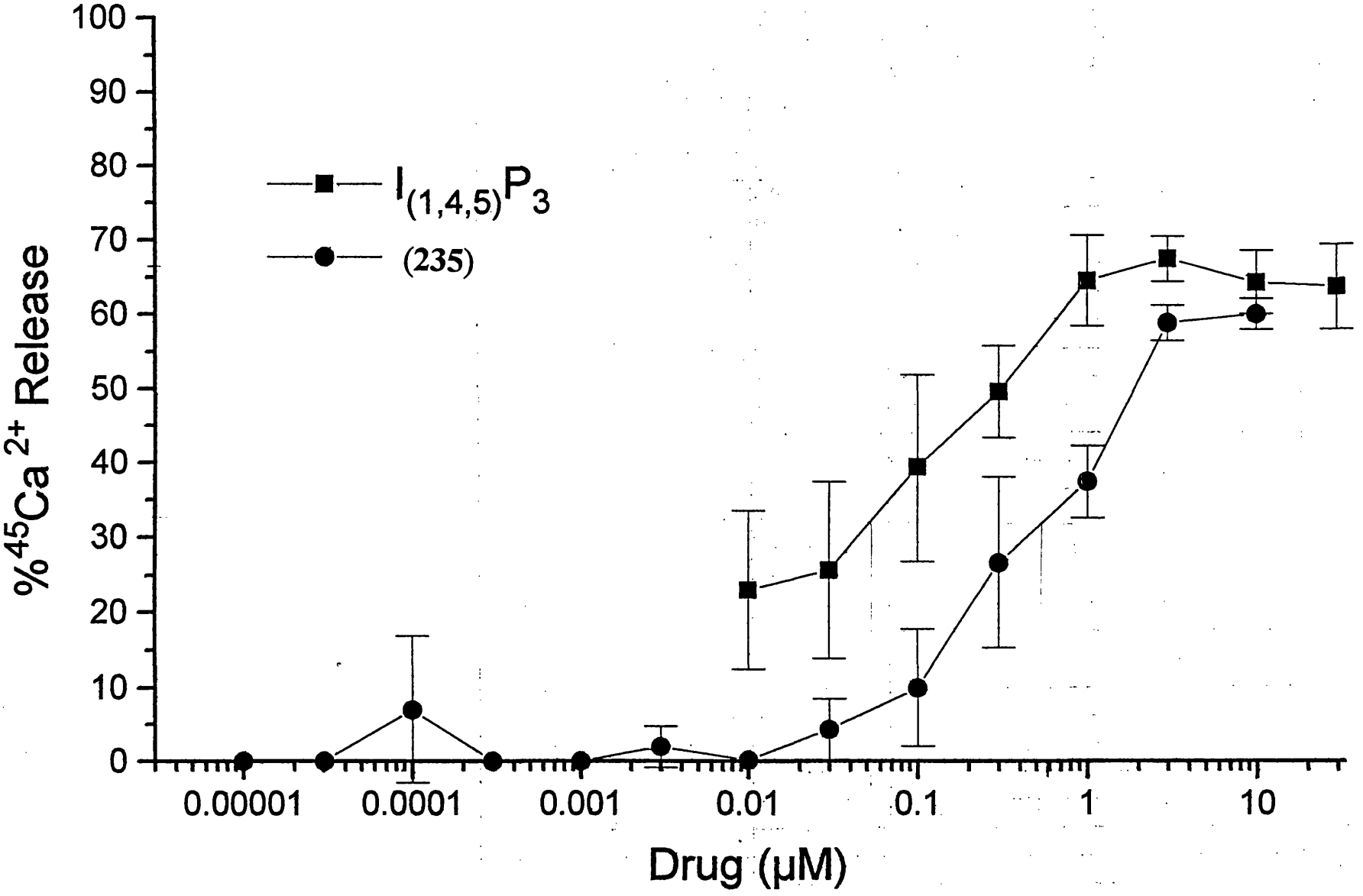


Fig. 5.8 $^{45}Ca^{2+}$ release from permeabilised platelets induced by Ins(1,4,5)P_3 and (235) (n=3).

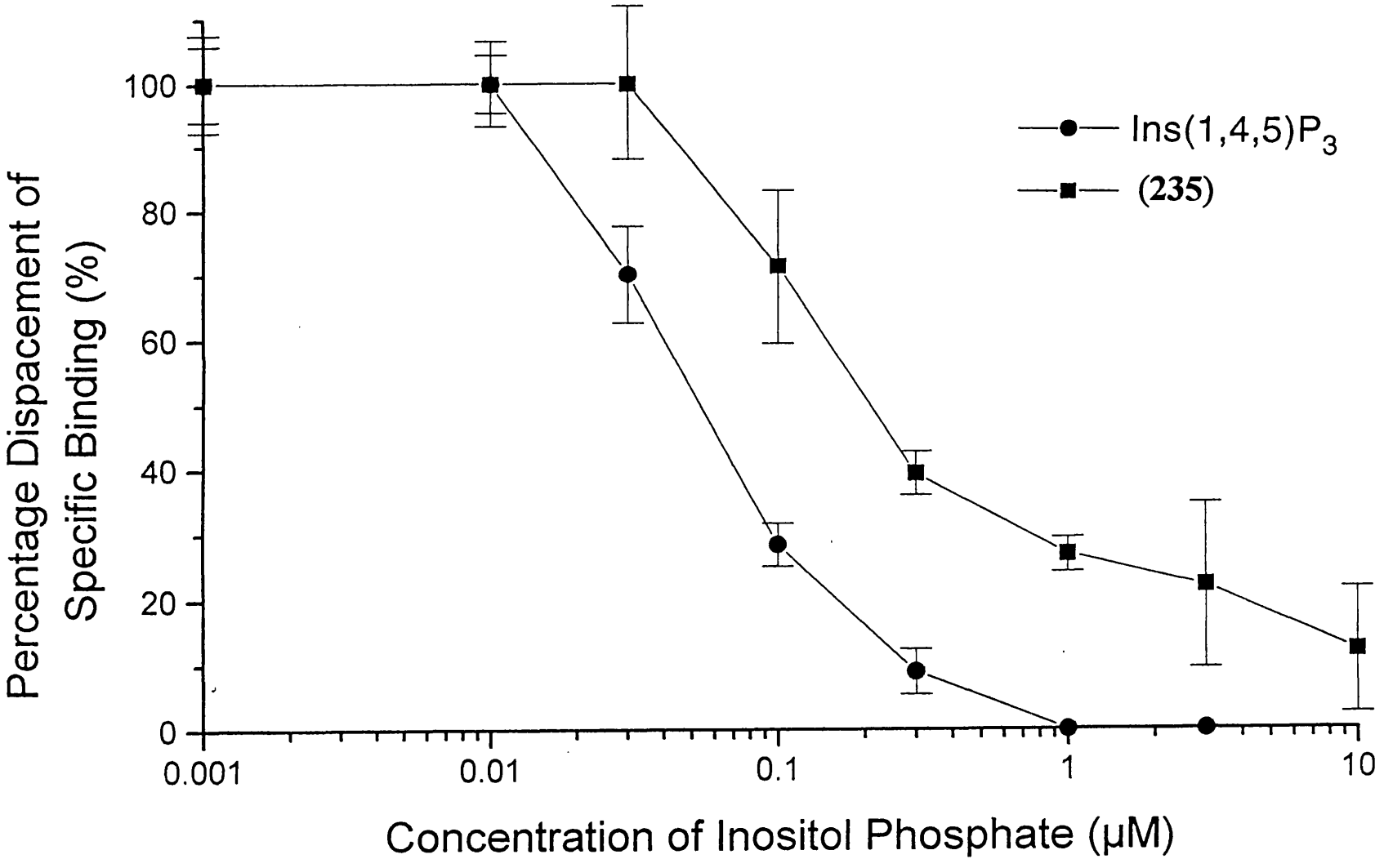


Fig. 5.9 Displacement of specific [^{3}H]-Ins(1,4,5)P_3 binding in rat cerebellar membranes by Ins (1,4,5)P_3 and by **(235)**. Non-specific binding was defined as binding not displaced by 10μM Ins(1,4,5)P_3 (n=3).

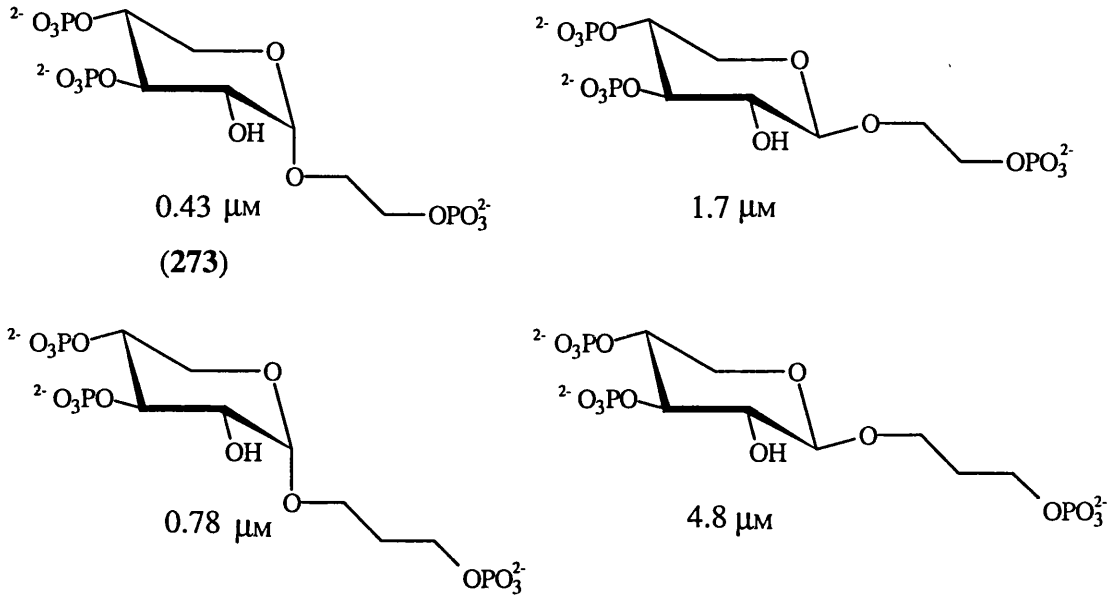


Fig. 5.10 EC_{50} values for Ca^{2+} mobilisation in permeabilised hepatocytes (taken from Moitessier *et al.*[302])

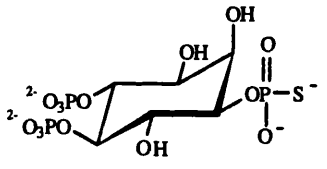
The second finding of interest is the relative Ca^{2+}-mobilising potencies of a series of both α- and β-anomeric (2-hydroxyethyl) and (3-hydroxypropyl) xylopyranoside trisphosphates.[302] Their structures and EC_{50} values for Ca^{2+}-release in permeabilised hepatocytes are given in fig. 5.10. It is noteworthy that (**273**), which represents the derivative of (**235**) in which the hydroxymethyl group has been deleted, and which of the four xylopyranosides most closely resembles the adenophostins, is the most potent. Increasing the aglycon chain length by one carbon atom approximately halved potency, while inversion of configuration at the anomeric centre reduced it approximately four-fold. Given that the hydroxymethyl group of (**235**) may engender a favourable ligand-receptor interaction (*vide ultra*), it would be interesting to compare (**235**) and (**273**) in the same system.

The sample of (**235**) prepared by Gigg *et al.* was found to be resistant to catabolism by both 5-phosphatase and 3-kinase, being neither a substrate nor an inhibitor of either of these enzymes.[274]

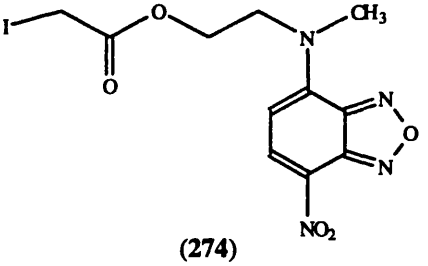
5.5 Preparation of a Fluorescent Label Based Upon (2-Hydroxy-ethyl) α-D-glucopyranoside 2',3,4-trisphosphate

The metabolic resistance of (**235**) made it an ideal compound onto which to attach a fluorescent label. Of particular interest was the possibility of preparing a probe to carry out fluorescence resonance energy transfer (FRET) experiments to study the purified Ins(1,4,5)P_3 receptor. This technique is based on the concept that the fluorescence emission of one molecule may excite the fluorescence of another, provided the two molecules are sufficiently close. The maximum distance (4–10nm) can only arise through specific binding; non-specifically bound molecules and non-bound molecules are too distant to allow the occurrence of FRET.

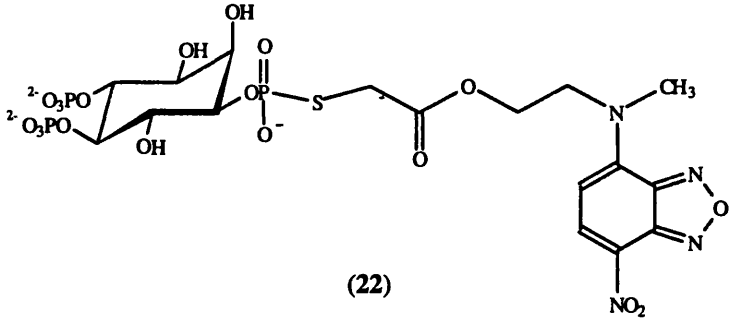
Fluorescently labelled compounds have been prepared previously by reaction of phosphorothioates with an iodinated derivative of the required label, for example DL-Ins(1,4,5)P_3-1S (**23**) with (**274**) to provide (**22**).[64]



(**23**)



(**274**)



(**22**)

It was reasoned that, as the 3,4-bisphosphate/2-hydroxy triad of (**235**) is essential for binding, the primary phosphate would be the ideal place onto which to append the label. Collaborators had previously used the BODIPY FL label,[303] and consequently it was

decided to react iodoacetamide (**275**; fig. 5.11) with (2-hydroxyethyl) α-D-glucopyranoside 3,4-bisphosphate-2'-phosphorothioate (**276**) in order to prepare the required (**277**). A route to (**276**) was designed.

Allyl 2,6-di-*O*-benzyl-α-D-glucopyranoside (**265**) was phosphitylated with tetrazole-activated bis(*p*-chlorobenzyloxy)(diisopropylamino)phosphine, then oxidised with MCPBA to give fully protected (**278**). The *p*-chlorobenzyl phosphate diester was chosen because of its increased acid stabilty compared to the benzyl diester.[242] This was considered important as the following step had produced acid strong enough to remove an isopropylidene acetal (*vide ultra*).

Intermediate (**278**) was converted to the corresponding (2-hydroxyethyl) glycoside (**279**) using osmium tetraoxide-sodium metaperiodate followed by sodium borohydride as described above. In this case a much longer reaction time was required and the intermediate aldehyde was purified by flash chromatography in an attempt to remove osmium tetraoxide. The structure of (**279**) was assigned largely on account of its ^{13}C NMR spectrum, which showed loss of allyl signals and presence of C-2' at 61.54ppm.

Thiophosphorylation of (**279**) was achieved using the method of Mills *et al.*[101] Thus, (**279**) was phosphitylated with tetrazole-activated bis(*p*-chlorobenzyloxy)-(diisopropylamino)phosphine in the usual way. The solvents were then evaporated and the monophosphite bisphosphate ester was treated with elemental sulphur in DMF-pyridine (2:1) to give (**280**), the sulphoxidation being complete within 30 min. The ^{1}H-coupled ^{31}P NMR spectrum of (**280**) in $CDCl_3$ (fig 5.12) exhibited the two protected ring phosphates as sextets at –2.33 and –1.89ppm, and the protected phosphorothioate as a septet at 68.76ppm.

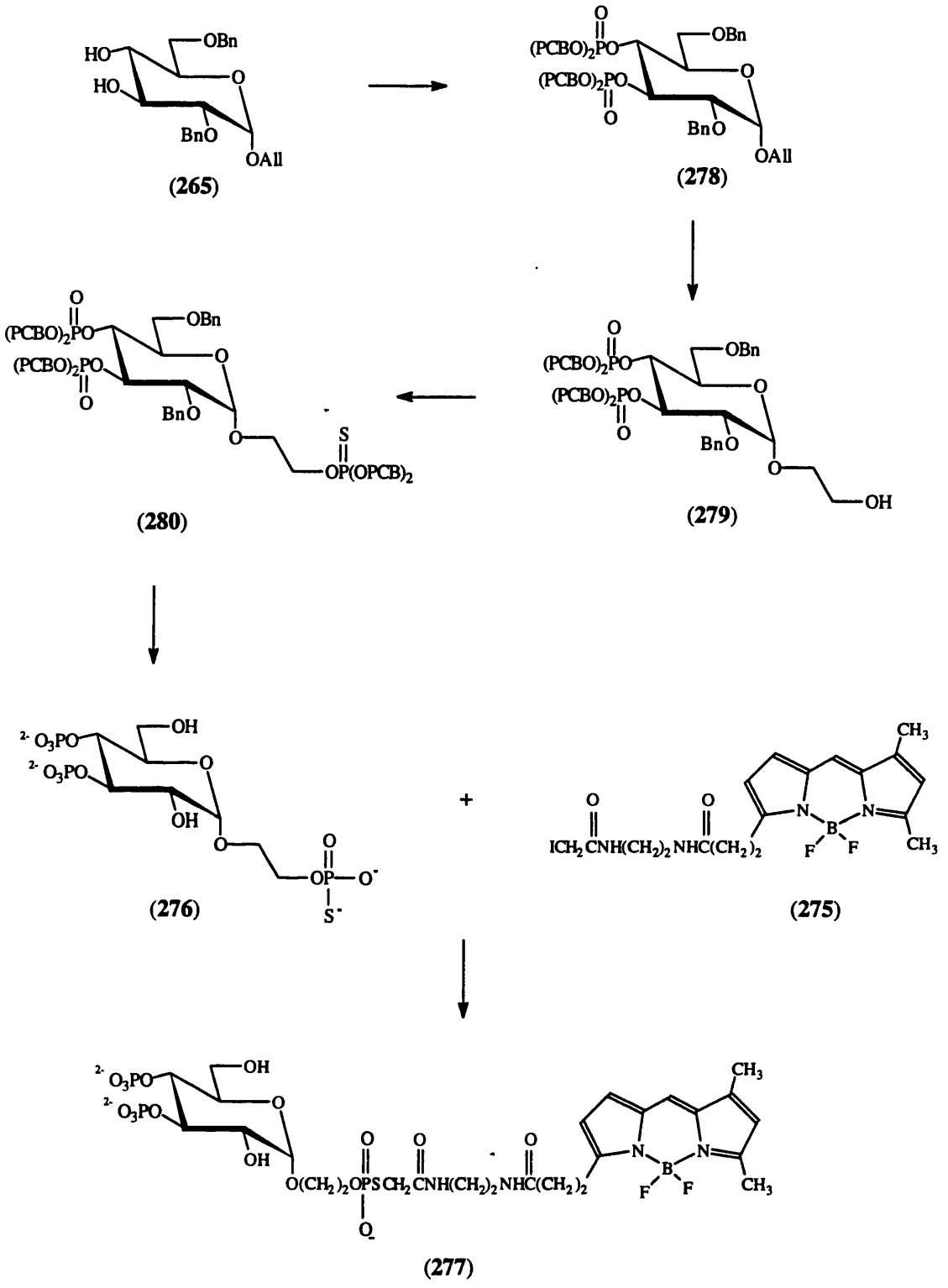
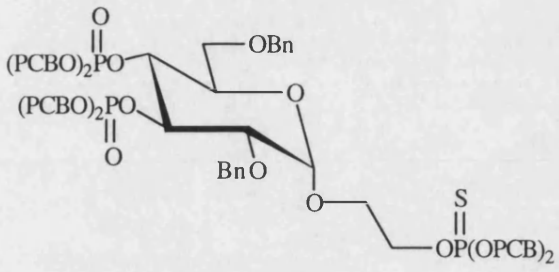


Fig. 5.11 Route to (277) from (265).



(**280**)

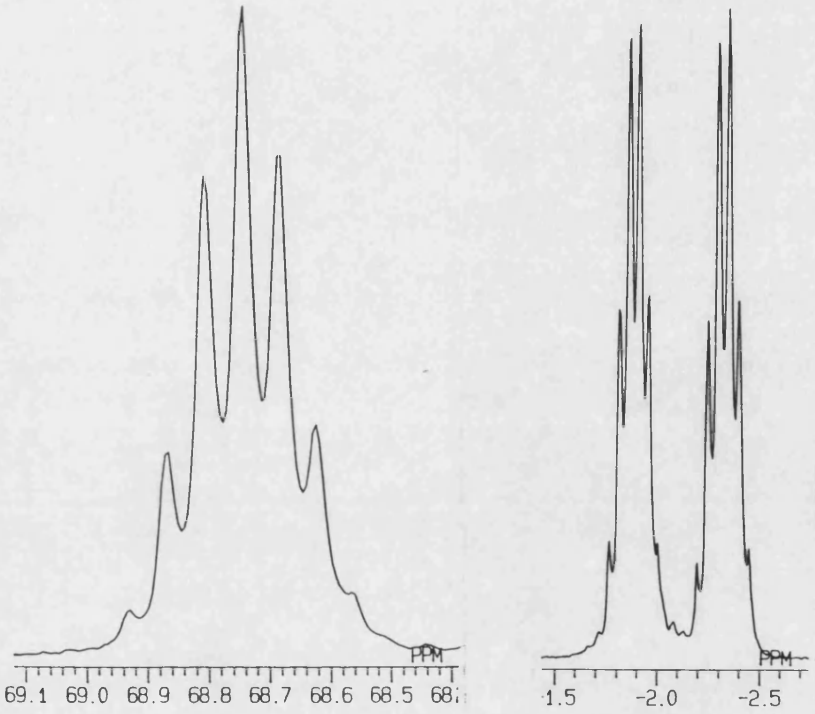
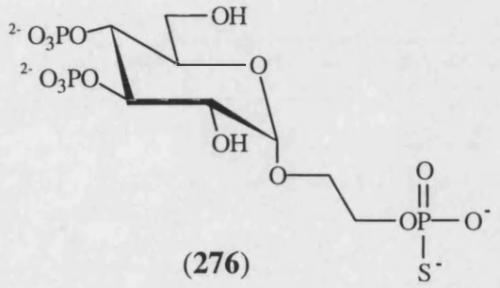


Fig. 5.12 162MHz ^{1}H-coupled ^{31}P NMR spectrum of (**280**) in $CDCl_3$. Different degrees of expansion have been used.

Deprotection of (**280**) with sodium in liquid ammonia provided (**276**), which was purified by ion-exchange chromatography and isolated as its triethylammonium salt. The ^{1}H-coupled ^{31}P NMR spectrum of (**276**) (fig 5.13) showed a triplet at 45.5ppm, corresponding to the primary phosphorothioate and two doublets at 1.43 and 0.94ppm, corresponding to the ring phosphates.



(**276**)

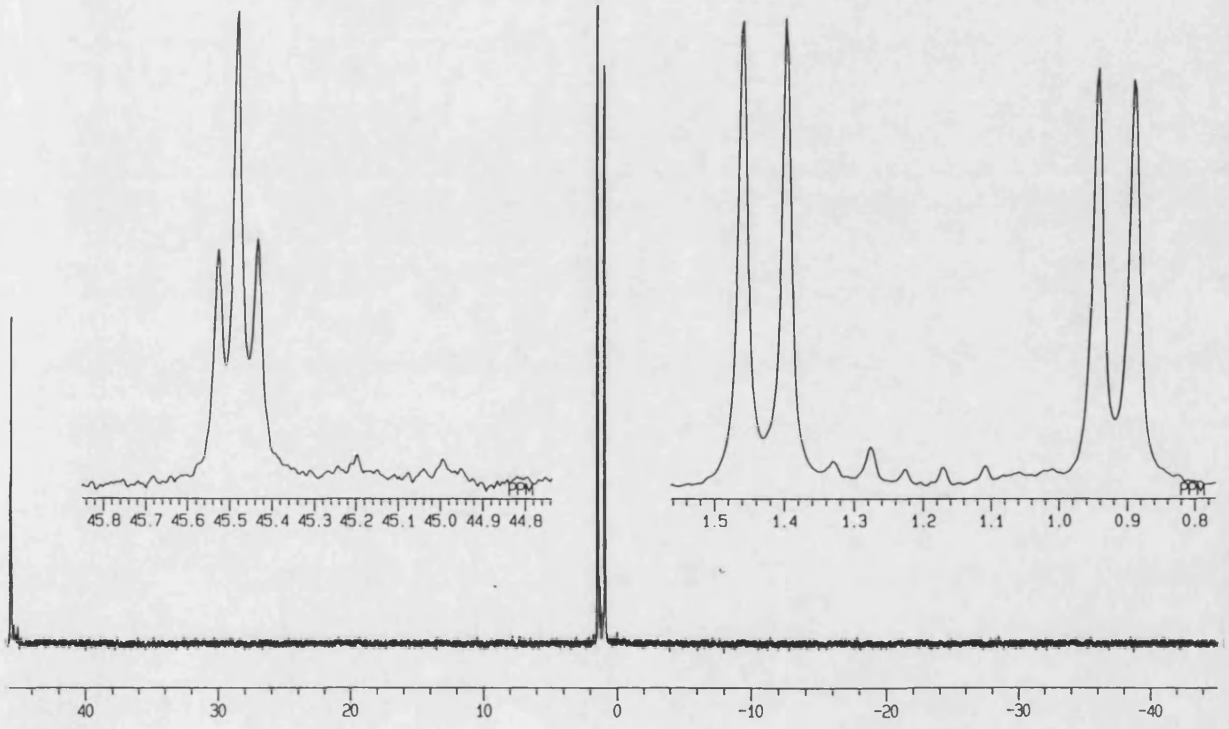
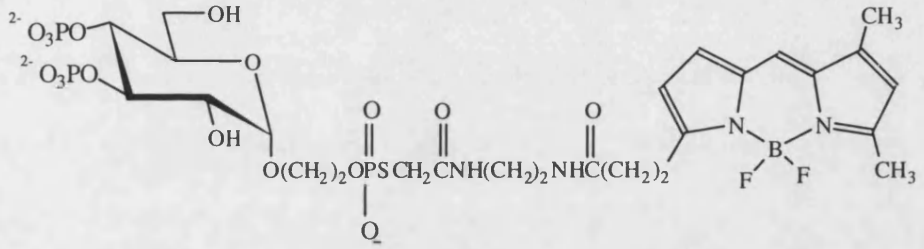


Fig. 5.13 162MHz ^{31}P NMR and (inset) ^{1}H-coupled ^{31}P NMR spectra of (**276**) in D_2O, pH *ca.* 4.

Phosphorothioate (**276**) was stirred with 1.2 equiv. of (**275**) in ethanol at room temperature. After two hours ^{31}P NMR spectroscopy indicated that the orange suspension contained a coupled product, judging by signals at δ_P 16.5, 1.3 and 0.9ppm. After ion-exchange chromatography, the ^{1}H-coupled ^{31}P NMR spectrum of (**277**; fig. 5.14) demonstrated the presence of the two ring phosphates, presenting as doublets, and the coupled phosphorothioate, presenting as a quintet. In addition, the ^{19}F NMR spectrum showed a peak at δ_F –122.6ppm and fluorescence spectra were obtained (fig. 5.15). However, the ^{31}P NMR spectrum clearly exhibits an impurity and the extremely poor yield of this reaction (*ca.* 10%) is cause for concern. Lack of time prevented optimisation of this strategy, but this will be investigated if encouraging results emerge from the preliminary FRET experiments.



(**277**)

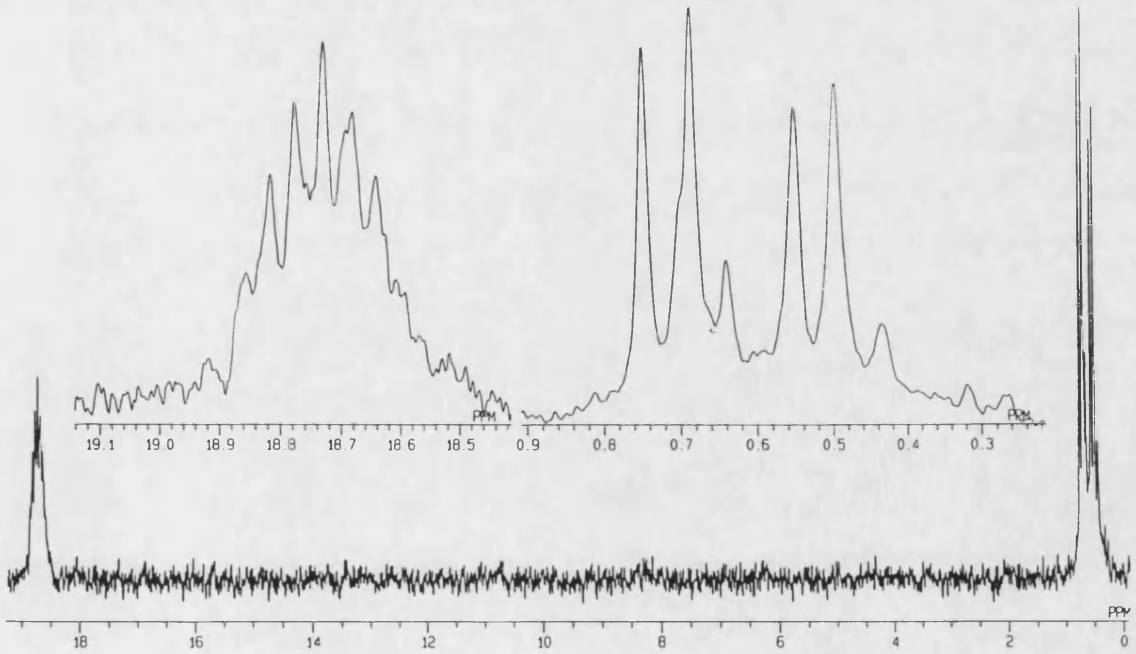


Fig. 5.14 162MHz ^{1}H-coupled ^{31}P NMR spectrum of (**277**) in D_2O, pH *ca.* 5.

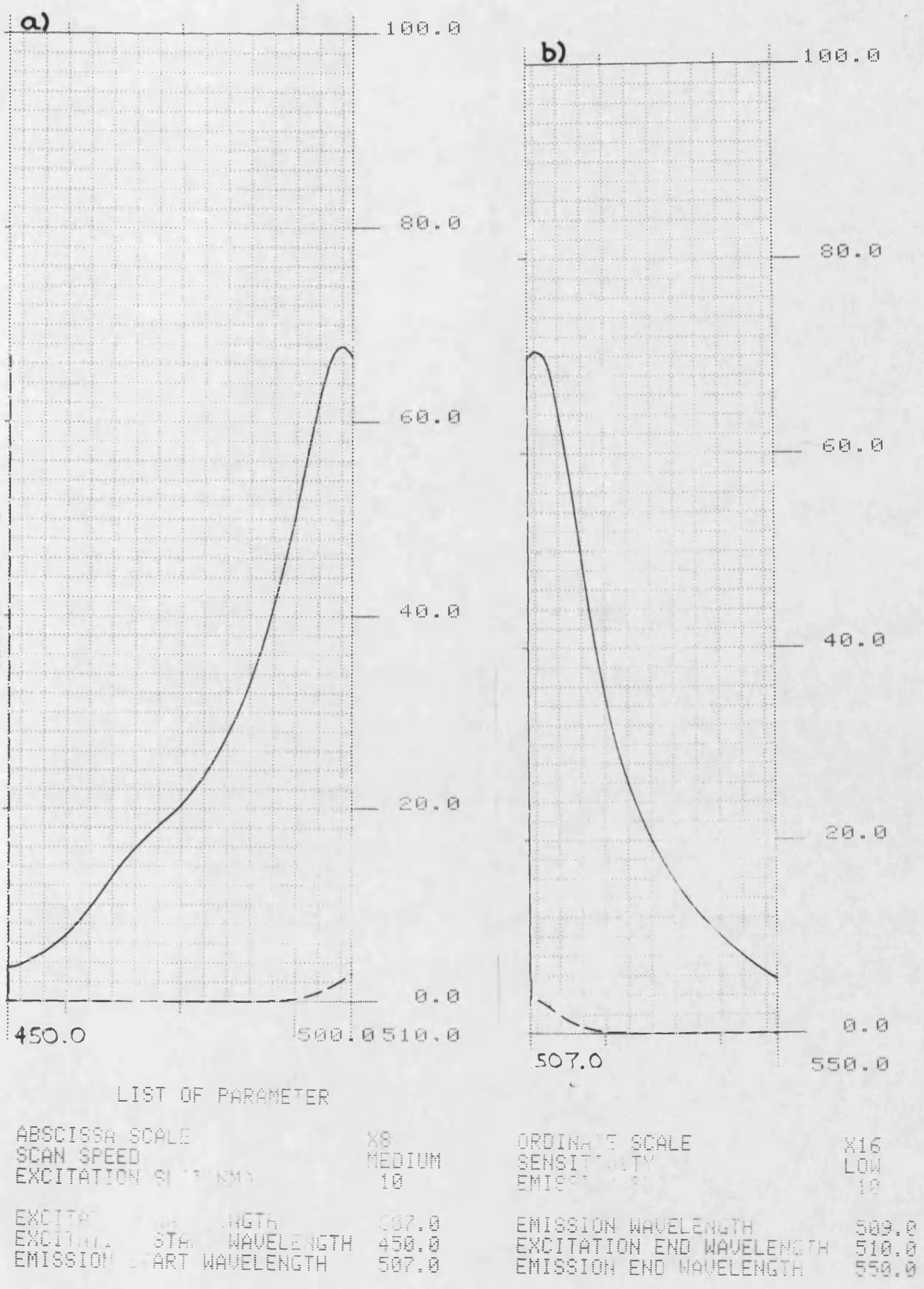


Fig. 5.15 a) Excitation and b) emission fluoresence spectra of (**277**) (—) and water (**---**).

CHAPTER SIX

EXPERIMENTAL

6.1 General Information

Thin layer chromatography (TLC) was performed on precoated plates (Merck TLC aluminium sheets silica 60 F_{254}, Art no. 5554): the compounds were detected by spraying with phosphomolybdic acid in methanol followed by heating, unless otherwise stated. Flash chromatography refers to the procedure developed by Still *et al.*,[304] and was carried out using Sorbsil C60 silica gel.

^{1}H and ^{13}C NMR spectra were recorded on either JEOL GX270 or 400 spectrometers. Unless otherwise stated, chemical shifts were measured in parts per million (ppm) relative to internal tetramethylsilane. ^{31}P NMR spectra were recorded on JEOL FX-90Q or GX-400 NMR spectrometers and ^{31}P NMR chemical shifts were measured in ppm relative to external 85% H_3PO_4. *J* values are given in Hz and the following abbreviations are used: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet, br, broad; exch., exchanged with. Proton assignments were established with 2D COSY experiments and the number of protons attached to carbon atoms was established by DEPT experiments. Where second order spectra do not deviate substantially from first order, the first order approximation has been used.

Infrared spectra were recorded on a Perkin-Elmer 782 spectrophotometer and were obtained on liquid films on NaCl plates or as KBr discs.

Melting points were determined using a Reichert-Jung Thermo Galen Kofler block and are uncorrected.

Microanalysis was carried out at the University of Bath Microanalysis Service.

Low resolution mass spectra were recorded at the University of Bath Mass Spectrometry Service using +ve and -ve fast atom bombardment (FAB) with *m*-nitrobenzyl alcohol (NBA) as the matrix. High resolution accurate mass spectra were recorded at the University of Bath Mass Spectrometry Service and at the EPSRC Mass Spectrometry Service, Swansea.

Optical rotations were measured at ambient temperature using an Optical Activity Ltd. AA-10 polarimeter in a cell volume of $1cm^3$ and specific rotations are given in 10^{-1} deg $cm^2\ g^{-1}$.

Ion-exchange chromatography was performed on an LKB-Pharmacia Medium Pressure Ion-Exchange Chromatograph using Sepharose Q fast flow resin and gradients of triethylammonium bicarbonate (TEAB) as eluent.

Synthetic phosphates were assayed by adaptations of the Briggs phosphate test[244] as follows. For the qualitative test, $200mm^3$ aliquots of the ion-exchange column fractions were pipetted into a test tube and the solutions were evaporated to dryness at 200°C. Four drops of concentrated sulphuric acid were added to each tube, which was then heated in an oven at 200°C for 90 min. The tubes were cooled to room temperature and water ($200mm^3$) was added with shaking to dissolve the residue. To each tube were added $400mm^3$ of a solution of ammonium molybdate (2.5g) in water ($20cm^3$) and concentrated sulphuric acid ($8cm^3$), followed by $200mm^3$ of a solution of quinol (100mg) in water ($20cm^3$) and one drop of concentrated sulphuric acid, then finally $200mm^3$ of a solution of sodium sulphite (4g) in water ($20cm^3$). Each tube was heated at the boiling point of the mixture for 10 s. A blue colour indicated the presence of inorganic phosphate.

For the quantitative assay the mixtures treated as above were transferred to volumetric flasks and made up to $10cm^3$ with water. The UV absorbances at 340nm were recorded using $3cm^3$ quartz cells. Concentration was calculated from a standard curve compiled from UV absorbance values of known concentrations of KH_2PO_4 treated as above and measured at 340nm.

Compounds containing phosphorothioates were assayed qualitatively by a modification of the Ellman test[305] for sulphydryl groups as follows. To $250mm^3$ aliquots of the ion-exchange column fractions was added $1cm^3$ of a buffered solution of Ellman's reagent [0.04% $^{w}/_{v}$ 5,5'-dithio-bis(*o*-nitrobenzoic acid) in 10mM Tris buffer, pH 8]. The fractions containing phosphorothioates were identified by their deep yellow colour.

Chemicals were purchased from the Aldrich and Sigma chemical companies.

Dioxane refers to 1,4-dioxane; ether refers to diethyl ether; hexane refers to *n*-hexane; light petroleum refers to the boiling range of 60–80°C. Evaporation refers to removal of solvent under reduced pressure.

Acetonitrile, boron trifluoride etherate, *t*-butanol, dichloromethane and toluene were dried over calcium hydride, distilled and stored over 4Å sieves. *N,N*-dimethylformamide (DMF) was dried over barium oxide, distilled under reduced pressure and stored over 4Å sieves. Dimethyl sulphoxide (DMSO) and triethylamine were purchased in anhydrous form. Methanol was dried by the method of Lund and Bjerrum.[306] Pyridine was dried over potassium hydroxide pellets, distilled and then stored over potassium hydroxide pellets. Tetrahydrofuran (THF) was dried by passing through activated alumina to expel peroxide radicals followed by distillation from sodium in the presence of benzophenone ketyl.

6.2 Synthesis of D-2-Deoxy-*myo*-Inositol-1,3,4,5-Tetrakisphosphate

6.2.1 Methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (150)

A 1 litre flask containing methyl α-D-glucopyranoside (**142**) (112g, 0.58mol), PTSA (2g), benzaldehyde dimethyl acetal (91cm^3, 0.61mol) and DMF (500cm^3) was fitted with an air condenser, attached to a water pump *via* a three-way tap and evacuated. The system was stirred at 70°C until methanol ceased to condense (4 h). The solution was cooled and concentrated to give a waxy white residue. Crystallisation from 2%$^{w}/_{v}$ aqueous $NaHCO_3$ solution (1200cm^3) gave the title compound as fine white needles (147g, 90%); m.p. 167–168°C [lit.[198] 167.5–168.5°C]; $[\alpha]_D$ +92.0 (*c* 5.0, $CHCl_3$) [lit.[198] +105].

6.2.2 Methyl 4,6-*O*-benzylidene-α-D-mannopyranoside (155)

A 1 litre flask containing methyl α-D-mannopyranoside (**153**) (105g, 0.54mol), PTSA (2g), benzaldehyde dimethyl acetal (85cm^3, 0.57mol) and DMF (500cm^3) was fitted with an air condenser, attached to a water pump *via* a three-way tap and evacuated. The system was stirred at 70°C until methanol ceased to condense (3.5 h). TLC (ethyl acetate : methanol 4:1) indicated a major product (R_f 0.58), a minor product (R_f 0.71) and unreacted starting material (R_f 0.25). The solution was cooled and concentrated. To the clear residue was added light petroleum (500cm^3) and 2%$^{w}/_{v}$ aqueous $NaHCO_3$ solution (1000cm^3). The mixture was vigorously shaken for 5 min, then stood at 0°C for 3 h. The white precipitate thus obtained was filtered and washed well with light petroleum. Recrystallisation from chloroform-toluene 1:9 (1000cm^3) gave the title compound as fluffy white crystals (73g, 48%); m.p. 145–146°C [lit.[208] 146–147°C]; $[\alpha]_D$ +35.1 (*c* 2.5, DMF) [lit.[208] +52].

6.2.3 Methyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (157), methyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (143) and methyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (156)

a) A solution of (**150**) (5.5g, 19.5mmol) in dry DMF (40cm^3) was stirred with sodium hydride (813mg of a 60%$^{w/w}$ dispersion in mineral oil, 19.5mmol) and benzyl bromide (2.4cm^3, 20.5mmol) for 2 h. Methanol (10cm^3) was added and stirring was continued for a further 15 min. The solvents were evaporated and the residue was extracted with ether (250cm^3). The organic extract was washed with water (100cm^3) and saturated aqueous NaCl solution (100cm^3), dried ($MgSO_4$), filtered and concentrated. The residual oil was subjected to flash chromatography (eluent hexane : ethyl acetate 7:3) to give (**157**) (1.9g, 21%); R_f 0.62 in hexane : ethyl acetate 3:2; m.p. 93–94°C (from ethanol) [lit.[307] 93°C]; $[\alpha]_D$ -29.6 (*c* 4.6, $CHCl_3$) [lit.[307] –32].

Further elution gave (**143**) (2.8g, 39%); R_f 0.50 in hexane : ethyl acetate 2:3; m.p. 128–130°C (ethyl acetate-hexane) [lit.[210] 129.5°C]; $[\alpha]_D$ +35.4 (*c* 5.0, $CHCl_3$) [lit.[210] +35].

Further elution gave (**156**) (1.3g, 19%); R_f 0.31 (hexane : ethyl acetate 2:3); m.p. 184–187°C (ethanol) [lit.[210] 185°C]; $[\alpha]_D$ +77.8 (*c* 5.0, $CHCl_3$) [lit.[210] +84].

b) A mixture of (**150**) (10.4g, 37.0mmol) and dibutyltin oxide (10.1g, 40.7mmol) in toluene (300cm^3) was heated under reflux for 3 h with continuous azeotropic removal of water (Dean-Stark trap). The solution was cooled and concentrated. To the white residue thus obtained was added dry DMF (30cm^3) and benzyl bromide (6.4cm^3, 53.8mmol). This mixture was stirred at 100°C for 3 h, then cooled. The solvent was evaporated and the residue was stirred with ether (500cm^3) and saturated aqueous $NaHCO_3$ solution (200cm^3) for 1 h. The resulting suspension was filtered through Celite, the organic layer was collected, dried ($MgSO_4$), filtered and concentrated. The orange oil thus obtained was subjected to flash chromatography (eluent hexane : ethyl acetate 7:3) to give (**143**) (6.3g, 46%) and (**156**) (2.7g, 19%).

c) The method employed is a modification of that described by Corrie.[216] A mixture of (**150**) (9.7g, 34.2mmol), dibutyltin oxide (9.1g, 36mmol), tetrabutylammonium iodide

(12.7g, 34.2mmol), benzyl bromide (4.5cm^3, 37.7mmol) and acetonitrile (400cm^3) was heated under reflux for 16 h *via* a Soxhlet thimble containing 4Å molecular sieves. The solution was cooled and concentrated. Purification and flash chromatography as above gave (**143**) (6.2g, 49%) and (**156**) (1.6g, 13%).

d) The method employed is a modification of that described by Boons *et al.*[217] A mixture of (**150**) (4.9g, 17.4mmol) and dibutyltin dimethoxide (4.1ml, 19.5mmol) was heated under reflux in dry toluene (300cm^3) for 1 h under Dean-Stark conditions. The solution was cooled to room temperature. Tetrabutylammonium iodide (9.7g, 26.1mmol) and benzyl bromide (2.3cm^3, 19.1mmol) were added and the mixture was stirred at 50°C under an atmosphere of nitrogen for 48 h. The solution was cooled and concentrated. Purification and flash chromatography as above gave (**143**) (2.6g, 41%) and (**156**) (1.0g, 15%).

e) The method employed is a modification of that described by Lampe *et al.*[64] Compound (**150**) (11.3g, 39.9mmol) was stannylated as in method *b)*. To the concentrated residue were added caesium fluoride (15.2g, 99.8mmol, dried *in vacuo* over P_2O_5 at 60°C for 24 h), dry DMF (40cm^3) and benzyl bromide (5.2cm^3, 43.9mmol). This suspension was stirred at room temperature under an atmosphere of nitrogen for 16 h. Purification and flash chromatography as above gave (**143**) (3.7g, 25%) and (**156**) (7.7g, 52%).

6.2.4 Methyl 2-*O*-allyl-4,6-*O*-benzylidene-α-D-glucopyranoside (161) and methyl 3-*O*-allyl-4,6-*O*-benzylidene-α-D-glucopyranoside (162)

Analogous treatments of the stannylene (**158**) with allyl bromide gave (**161**), R_f 0.63 (ethyl acetate); m.p. 118.5–120°C (ethyl acetate-hexane) [lit.[308] 115–116°C]; $[\alpha]_D$ +70.0 (*c* 1.5, $CHCl_3$) [lit.[308] +75.8]; and (**162**), R_f 0.53 (ethyl acetate); m.p. 155°C [lit.[308] 154–155°C]; $[\alpha]_D$ +100.7 (*c* 1.5, $CHCl_3$) [lit.[308] +104].

6.2.5 Methyl 4,6-*O*-benzylidene-2-*O*-(*p*-methoxybenzyl)-α-D-glucopyranoside (163) and methyl 4,6-*O*-benzylidene-3-*O*-(*p*-methoxybenzyl)-α-D-glucopyranoside (164)

Analogous treatments of the stannylene (**158**) with *p*-methoxybenzyl chloride gave (**163**), R_f 0.44 (hexane : ethyl acetate 2:3); m.p. 102°C (ethanol); $[\alpha]_D$ +38.6 (*c* 1.4, acetone); (Found: C, 65.8; H, 6.56. Calc. for $C_{22}H_{26}O_7$: C, 65.64; H, 6.52%); δ_H ($CDCl_3$; 270MHz) 2.75 (1 H, d, *J* 2.2, exch. D_2O, OH), 3.35 (3 H, s, OCH_3), 3.40–3.50 (2 H, m, 2-H, 4-H), 3.64–3.84 (5 H, m, 5-H, 6-H_{ax}, $ArOCH_3$), 4.12 (1 H, td, *J* 2.2, 9.3, simplifies to t on D_2O exch., 3-H), 4.25 (1 H, dd, *J* 4.4, 9.7, 6-H_{eq}), 4.55 (1 H, d, *J* 3.5, 1-H), 4.62, 4.69 (2 H, AB, J_{AB} 11.9, ArCH_2O), 5.50 (1 H, s, 7-H), 6.88 (2 H, d, *J* 8.6, 3-H and 5-H of *p*-methoxybenzyl ring), 7.24–7.50 (7 H, m, aromatic CH); δ_H ($CDCl_3$, 67.8MHz) 55.14, 55.22 (2 x OCH_3), 61.90 (CH), 68.84 (6-C), 69.91 (CH), 72.82 (ArCH_2O), 79.06, 81.14 (2 x CH), 98.57 (1-C), 101.81 (7-C), 113.83 (3-C and 5-C of *p*-methoxybenzyl ring), 126.83, 128.83, 129.03, 129.64 (aromatic CH), 129.87 (1-C of *p*-methoxybenzyl ring), 137.02 (1-C of benzyl ring), 159.39 (4-C of *p*-methoxybenzyl ring); *m/z*: (FAB^+) 403 [$(M+1)^+$, 5%];

and (**164**), R_f 0.25 (hexane : ethyl acetate 2:3); m.p. 174–175.5°C (ethanol); $[\alpha]_D$ +45.8 (*c* 1.2, acetone); (Found: C, 65.6; H, 6.53. Calc. for $C_{22}H_{26}O_7$: C, 65.64; H, 6.52%); δ_H ($CDCl_3$, 400MHz) 2.42 (1 H, d, *J* 7.3, exch. D_2O, OH), 3.43 (3 H, s, OCH_3), 3.59–3.94 (8 H, m, 2-H, 3-H, 4-H, 5-H, 6-H_{ax}, $ArOCH_3$), 4.28 (1 H, dd, *J* 4.4, 9.9, 6-H_{eq}), 4.71 (1 H, d, J_{AB} 11.0, ArC*H*HO), 4.77 (1 H, d, *J* 4.0, 1-H), 4.87 (1 H, d, J_{AB} 11.0, ArCH*H*O), 5.56 (1 H, s, 7-H), 6.84 (2 H, d, *J* 8.5, 3-H and 5-H of *p*-methoxybenzyl ring), 7.24–7.51 (7 H, m, aromatic CH); δ_H ($CDCl_3$, 100MHz) 55.23, 55.36 (2 x OCH_3), 62.58 (CH), 69.00 (6-C), 72.31 (CH), 74.47 (ArCH_2O), 78.38, 81.95 (2 x CH), 99.89 (1-C), 101.26 (7-C), 113.79 (3-C and 3-C of *p*-methoxybenzyl ring), 126.02, 128.24, 128.95, 129.70 (aromatic CH), 130.54 (1-C of *p*-methoxybenzyl ring), 137.38 (1-C of benzyl ring), 159.25 (4-C of *p*-methoxybenzyl ring); *m/z*: (FAB^+) 403 [$(M+1)^+$, 5%].

6.2.6 Methyl 2-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranoside (159)

A mixture of (**150**) (5.0g, 17.7mmol) and dibutyltin dimethoxide (4.1cm^3, 19.5mmol) was heated under reflux in dry toluene (300cm^3) for 1 h under Dean-Stark conditions. The solution was cooled to 0°C under an atmosphere of nitrogen and triethylamine (0.1cm^3, 0.9mmol) was added. Benzoyl chloride (2.3cm^3, 19.5mmol) was added dropwise and the solution was stirred at room temperature for 1 h, when TLC (ethyl acetate : hexane 3:2) indicated a single product (R_f 0.74). The solution was stirred with methanol (5cm^3) for 5 min and the solvents were evaporated. The residue was dissolved in ether (300cm^3) and the solution was stirred with saturated aqueous $NaHCO_3$ solution (100cm^3) for 30 min. The resulting suspension was filtered through Celite and the organic layer was dried ($MgSO_4$), filtered and concentrated. The resulting oil was purified by flash chromatography (eluent hexane : ethyl acetate 7:3) to give exclusively the title compound (4.9g, 72%); m.p. 170–172°C (ethanol) [lit.[309] 169–170°C]; $[\alpha]_D$ +108.5 (*c* 1.2, $CHCl_3$) [lit.[309] +108].

6.2.7 Methyl 4,6-*O*-benzylidene-2-*O*-tosyl-α-D-glucopyranoside (160)

Compound (**150**) (500mg, 1.8mmol) was stannylated as above and cooled to 0°C. Triethylamine (0.01cm^3, 0.1mmol) and tosyl chloride (355mg, 1.8mmol) were added and the solution was stirred at room temperature for 16 h. The solvent was evaporated and the residue was subjected to flash chromatography (eluent hexane : ethyl acetate 7:3) to give exclusively the title compound (533mg, 69%); R_f 0.63 in hexane : ethyl acetate 2:3; m.p. 152–154°C (ethanol) [lit.[310] 153–154°C]; $[\alpha]_D$ +60.0 (*c* 0.8, $CHCl_3$) [lit.[310] +64.2].

6.2.8 Methyl 3-*O*-benzoyl-2-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (144)

A mixture of (**150**) (129g, 0.46mol) and dibutyltin oxide (125g, 0.50mol) was heated under reflux in toluene (1500cm^3) for 3 h with continuous azeotropic removal of water (Dean-Stark trap). The solution was cooled and concentrated to give a white solid to which were added acetonitrile (1200cm^3), tetrabutylammonium bromide (100g,

0.31mol) and benzyl bromide (60cm^3, 0.50mol). This mixture was heated under reflux for 2 days *via* a Soxhlet thimble containing 4Å molecular sieves. The solution was cooled, triethylamine (100cm^3) was added and stirring was continued for 3 h. The solvents were evaporated and to the residue ether (2000cm^3) and saturated aqueous $NaHCO_3$ solution (700cm^3) were added. The mixture was vigorously stirred for 1 h and the resultant suspension was filtered through Celite. The residue was well washed with ether (250cm^3) and the combined organic fraction was dried ($MgSO_4$), filtered and evaporated. This crude mixture of monobenzyl ethers was dissolved in pyridine (500cm^3) containing DMAP (1g). Benzoyl chloride (100cm^3, 0.86mol) was added dropwise at 0°C and the system was stirred at room temperature for 3 h. Methanol (200cm^3) was added and stirring was continued for a further 30 min. The solvents were evaporated and co-evaporated with toluene (3 x 500cm^3). The orange residue thus obtained was triturated with ether (3 x 500cm^3) and the combined organic extracts were washed with 5M aqueous HCl (2 x 250cm^3) and water (4 x 250cm^3), dried ($MgSO_4$), filtered and concentrated to give a pale yellow syrup. The title compound was obtained by fractional crystallisation from ethanol (74g, 34%); R_f 0.57 (hexane : ethyl acetate 3:2); m.p. 136–137°C (ethanol); $[\alpha]_D$ –6.3 (*c* 4.4, $CHCl_3$); (Found: C, 70.3; H, 5.82. Calc. for $C_{28}H_{28}O_7$: C, 70.56; H 5.93%); δ_H ($CDCl_3$; 270 MHz) 3.43 (3 H, s, OCH_3), 3.66–3.79 (3 H, m, 2-H, 4-H, 6-H_{ax}), 3.97 (1 H, td, $J_{5\text{-H, 6-Hax}} = J_{5\text{-H, 4-H}} = 9$, $J_{5\text{-H, 6-Heq}}$ 4.5, 5-H), 4.30 (1 H, dd, *J* 4.5, 10.5, 6-H_{eq}), 4.61, 4.61 (2 H, AB, J_{AB} 12.7, PhCH_2O), 4.75 (1 H, d, *J* 3.8, 1-H), 5.46 (1 H, s, 7-H), 5.85 (1 H, t, *J* 9, 3-H), 7.16–7.61 (13 H, m, aromatic CH), 8.02–8.07 (2 H, m, 2-H; δ_C ($CDCl_3$; 67.8 MHz) 55.45 (OCH_3), 62.44 (CH), 69.01 (6-C), 71.23 (CH), 72.90 (PhCH_2O), 79.69 (2-C), 98.81 (1-C), 101.44 (7-C), 126.14, 127.91, 127.96, 128.10, 128.28, 128.40, 128.88, 129.81 (aromatic CH), 130.26 (1-C of benzoyl ring), 132.87 (4-C of benzoyl ring), 137.04, 137.64 (2 x 1-C of phenyl rings), 165.37 (PhCO_2); *m/z* (CI) 477 [$(M+1)^+$, 10%]; ν_{max} 1730cm^{-1}.

6.2.9 Methyl 3-*O*-benzoyl-2-*O*-benzyl-α-D-glucopyranoside (167)

A solution of (**144**) (12.9g, 27.2mmol) in 80%$^{v/v}$ acetic acid in water (100cm^3) was heated under reflux for 1.75 h. The solution was cooled, the solvents evaporated and co-evaporated with toluene (3 x 60cm^3). The white residue thus obtained was extracted

with ethyl acetate (3 x 100cm^3). The combined extracts were washed with water (50cm^3), dried ($MgSO_4$), filtered and concentrated to give the title compound as a white solid of sufficient purity to be used for the next step (10.4g, 98%). A sample was crystallised twice from isopropanol to provide an analytically pure sample; R_f 0.17 (hexane : ethyl acetate 2:3); m.p. 114–115°C; $[\alpha]_D$ +97.7 (*c* 1.1, $CHCl_3$); (Found: C, 64.6; H, 6.22. Calc. for $C_{21}H_{24}O_7$: C, 64.92; H, 6.23%);δ_H ($CDCl_3$; 270MHz) 2.60 (1 H, t, *J* 5.6, exch. D_2O, 6-OH), 3.39 (3 H, s, OCH_3), 3.54 (1 H, d, *J* 4.6, exch. D_2O, 4-OH), 3.61 (1 H, dd, *J* 3.7, 9.6, 2-H), 3.69–3.75 (2 H, m, 4-H, 5-H), 3.83 (2 H, br s, 6-H, 6-H'), 4.60, 4.60 (2 H, AB, J_{AB} 12.2, PhCH_2O), 4.72 (1 H, d, *J* 3.7, 1-H), 5.50 (1 H, br t, *J* 9.3, 3-H), 7.23–7.62 (8 H, m, aromatic CH), 7.98–8.01 (2 H, m, 2-H and 6-H of benzoyl ring); δ_C ($CDCl_3$; 67.8MHz) 55.25 (OCH_3), 61.71 (6-C), 69.67, 71.22 (CH), 72.77(PhCH_2O), 75.75, 76.66 (CH), 97.73 (1-C), 127.86, 128.33 (aromatic CH), 129.69 (1-C of benzoyl ring), 129.82 (aromatic CH), 133.21 (4-C of benzoyl ring), 137.57 (1-C of benzyl ring), 167.35 (PhCO_2); *m/z* (FAB$^-$) 387 [(M-1)$^-$, 30%].

6.2.10 Methyl 3,4-di-*O*-benzoyl-2-*O*-benzyl-6-bromo-6-deoxy-α-D-glucopyranoside (145), methyl 3,4-di-*O*-benzoyl-6-bromo-6-deoxy-α-D-glucopyranoside (165) and methyl 3-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranoside (166)

a) From (144) by Hanessian–Hullar reaction. The method employed is a modification of that described by Hanessian.[311] Carbon tetrachloride (50cm^3) was passed through activated alumina and collected in a dry 100cm^3 round bottomed flask containing a mixture of (**144**) (2.0g, 4.2mmol), barium carbonate (1.2g, 6.3mmol, dried *in vacuo* over P_2O_5 at 75°C for 16 h) and *N*-bromosuccinimide (897mg, 5.0mmol, dried as for barium carbonate). This mixture was heated under reflux under an atmosphere of nitrogen for 1 h, when TLC indicated three major products and unreacted starting material. The suspension was cooled, filtered and the residue well washed with dichloromethane (100cm^3). The combined filtrates were washed with 5%$^{w}/_{v}$ aqueous $Na_2S_2O_3$ solution (100cm^3) and water (100cm^3), dried ($MgSO_4$), filtered and concentrated to give a colourless oil which was subjected to flash chromatography (eluent hexane : ethyl acetate 7:3) to give (**145**) (653mg, 28%); R_f 0.69 (hexane : ethyl acetate 2:3); m.p. 111°C (ethanol); $[\alpha]_D$ –26.0 (*c* 2.5, $CHCl_3$); (Found: C, 60.7; H 4.80. Calc. for

$C_{28}H_{27}O_7Br$: C, 60.64; H, 4.91%); δ_H ($CDCl_3$; 400 MHz) 3.43 (1 H, ABX, $^2J_{AB}$ 11.3, ^3J 7.6, 6-H), 3.51 (3 H, s, OCH_3), 3.51 (1 H, ABX, $^2J_{AB}$ 11.3, ^3J 2.7, 6-H'), 3.76 (1 H, dd, *J* 3.7, 10.0, 2-H), 4.18 (1 H, ddd, *J* 2.4, 7.3, 9.8, 5-H), 4.59, 4.63 (2 H, AB, J_{AB} 12.5, PhCH_2O), 4.81 (1 H, d, *J* 3.7, 1-H), 5.28 (1 H, t, *J* 9.8, 4-H), 5.94 (1 H, t, *J* 9.8, 3-H), 7.20–7.61 (11 H, m, aromatic CH), 7.89–7.93 (4 H, m, 2-H and 6-H of benzoyl rings); δ_C ($CDCl_3$; 100.4 MHz) 31.71 (6-C), 55.74 (OCH_3), 69.04, 71.73, 71.76 (CH), 73.10 (PhCH_2O), 76.90 (CH), 97.91 (1-C), 128.00, 128.29, 128.46 (aromatic CH), 128.77 (1-C of benzoyl ring), 129.63, 129.74 (aromatic CH), 129.90 (1-C of benzoyl ring), 133.03, 133.52 (2 x 4-C of benzoyl rings), 137.42 (1-C of benzyl ring), 165.53 (PhCO_2); *m/z* (FAB$^+$) 555, 557 [(M+1)$^+$, 35%].

Further elution gave starting material (326mg, 16%).

Further elution gave (**165**) as a pale yellow oil (310mg, 16%); R_f 0.48 (hexane : ethyl acetate 2:3); $[\alpha]_D$ +30.9 (*c* 1.4, $CHCl_3$) [lit.[227] +32.8 and +34.4]; δ_H ($CDCl_3$; 270MHz) 2.48 (1 H, br s, exch. D_2O, 2-OH), 3.43–3.54 (2 H, m, 6-H, 6-H'), 3.57 (3 H, s, OCH_3), 3.91 (1 H, br dd, sharpens D_2O exch., *J* 3.7, 9.7, 2-H), 4.19 (1 H, ddd, *J* 3.7, 9.7, 10.6, 5-H), 4.95 (1 H, d, *J* 3.7, 1-H), 4.96 (1 H, t, *J* 9.7, 4-H), 5.36 (1 H, t, *J* 9.7, 3-H), 7.26–7.95 (10 H, m, aromatic CH); *m/z* (FAB$^+$) 465, 467 [(M+1)$^+$, 5%].

Further elution gave (**166**) (158mg, 10%); R_f 0.40 (hexane : ethyl acetate 2:3); m.p. 215–220°C (ethanol) [lit.[228] 217–218°C]; $[\alpha]_D$ +19.5 (*c* 1.3, $CHCl_3$) [lit.[228] +33.5].

b) The method employed is a modification of that described by Smith *et al.*[231] A solution of (**167**) (15.4g, 39.7mmol) in freshly distilled, dry THF (250cm^3) at 0°C under an atmosphere of nitrogen was sequentially treated with triphenylphosphine (10.9g, 41.7mmol) and freshly sublimed carbon tetrabromide (15.8g, 47.6mmol). The mixture was stirred for 1 h at 0°C and for 16 h at room temperature, when TLC (ethyl acetate) indicated consumption of starting material (R_f 0.38) to give a product (R_f 0.73). The solvent was evaporated and the residue was subjected to flash chromatography (eluent hexane : ethyl acetate 1:1) to remove triphenylphosphine oxide (R_f 0.30 in ethyl acetate). The crude product was dissolved in dry pyridine (150cm^3) and stirred with DMAP (250mg) and benzoyl chloride (5.0cm^3, 43.1mmol) for 2 h. Methanol (5cm^3) was added

and stirring was continued for 5 min. The solvents were evaporated and co-evaporated with toluene (3 x 200cm^3). The residue thus obtained was vigorously shaken with ether (500cm^3) and the resulting suspension was filtered. The filtrate was washed with water (200cm^3), dried ($MgSO_4$), filtered and concentrated to give a pale yellow oil. Crystallisation from ethanol gave (**145**) (13.9g, 63%).

6.2.11 Methyl 3,4-di-*O*-benzoyl-2-*O*-benzyl-6-deoxy-α-D-*xylo*-hex-5-enopyranoside (146)

a) Silver fluoride method. The method employed is a modification of that described by Cheung *et al.*[232] To a mixture of (**145**) (2.1g, 3.8mmol) and silver (I) fluoride (5.3g, 22.7mmol, dried *in vacuo* over P_2O_5 at 75°C for 24 h) was added dry pyridine (30cm^3) under an atmosphere of nitrogen. The suspension was stirred vigorously in the dark for 48 h. The suspension was concentrated and co-evaporated with toluene (3 x 50cm^3) The residue was taken up in ether (200cm^3). The suspension was filtered through Celite and the filtrate was washed with 5%$^{w}/_{v}$ aqueous $Na_2S_2O_3$ solution (100cm^3) and water (100cm^3), dried ($MgSO_4$), filtered and concentrated. The orange oil thus obtained was purified by flash chromatography (eluent hexane : ethyl acetate 4:1) to give a pale yellow oil. Crystallisation from ethanol gave the title compound (561mg, 31%); R_f 0.69 (hexane : ethyl acetate 2:3); m.p. 102–104°C; $[\alpha]_D$ –32 (*c* 1.0, $CHCl_3$); (Found: C, 70.8; H, 5.45. Calc. for $C_{28}H_{26}O_7$: C, 70.86; H 5.53%); δ_H ($CDCl_3$; 270MHz) 3.51 (1 H, s, OCH_3), 3.88 (1 H, dd, *J* 3.3, 9.9, 2-H), 4.62 (1 H, t, $^4J_{4\text{-H, 6-H}} = {}^2J_{6\text{-H, 6-H'}}$ =2.0, 6-H), 4.64, 4.66 (2 H, AB, J_{AB} 12.6, PhCH_2O), 4.79 (1 H, t, $^4J_{4\text{-H, 6-H'}} = {}^2J_{6\text{-H, 6-H'}}$ =2.0, 6-H'), 4.85 (1 H, d, *J* 3.3, 1-H), 5.77 (1 H, dt, ^3J 9.7, ^4J 2.0, 4-H), 5.96 (1 H, t, *J* 9.7, 3-H), 7.20–7.54 (11 H, m, aromatic CH), 7.93–8.03 (4 H, m, 2-H and 6-H of benzoyl rings); δ_C ($CDCl_3$; 67.8 MHz) 55.71 (OCH_3), 70.21, 71.47 (CH), 73.08 (Ph*C*H_2O), 76.53 (CH), 97.34 (6-C), 98.86 (1-C), 127.94, 127.99, 128.26, 128.41 (aromatic CH), 128.96, 129.66 (2 x 1-C of benzoyl rings), 129.71, 129.90 (aromatic CH), 132.96, 133.37 (2 x 4-C of benzoyl rings), 137.46 (1-C of benzyl ring), 150.30 (5-C), 165.32, 165.35 (2 x Ph*C*O_2); *m/z* (FAB$^+$) 475 [(M+1)$^+$, 5%]; ν_{max} 1730, 1670 cm^{-1}.

b) Iodide exchange in DMSO/DBU method. The method is a modification of that described by Sato *et al.*[233] A mixture of (**145**) (20.9g, 37.7mmol), sodium iodide (23.2g,

154.8mmol, dried *in vacuo* over P_2O_5 at 75°C for 2 h), tetrabutylammonium iodide (5.8g, 15.7mmol, dried as for sodium iodide) and dry DMSO (90cm^3) was stirred at 100°C under a stream of nitrogen for 2 h in the presence of 4Å sieves (about 50 pieces). DBU (11.2cm^3, 75.3mmol) was added to the orange solution which rapidly turned dark brown. Vigorous stirring was continued for 2 h, then the system was cooled. The resultant dark brown semi-solid mass was extracted with ether (3 x 500cm^3) and the combined extracts were washed with 5%$^{w}/_{v}$ aqueous $Na_2S_2O_3$ solution (300cm^3) and saturated aqueous KCl solution (500cm^3), dried ($MgSO_4$), filtered and concentrated. The concentrate was purified by flash chromatography (hexane : ethyl acetate 4:1) to give crude (**146**) as a pale yellow oil; crystallisation from ethanol gave pure (**146**) (11.0g, 62%).

6.2.12 (2*S*, 3*R*, 4*S*, 5*R*)-2,3-dibenzoyloxy-4-benzyloxy-5-hydroxycyclohexanone (147) and (2*S*, 3*R*, 4*S*, 5*S*)-2,3-dibenzoyloxy-4-benzyloxy-5-hydroxycyclohexanone (148)

a) By stoichiometric Ferrier rearrangement using mercury (II) trifluoroacetate. To a solution of (**146**) (4.6g, 9.7mmol) in acetone : water (5:2) containing 1% acetic acid (200cm^3) was added mercury (II) trifluoroacetate (4.3g, 10.2mmol). After 10 min the solution had assumed a pale lilac colour and TLC (ethyl acetate : hexane 3:2) indicated that all starting material (R_f 0.65) had been consumed to give an ill-defined product (R_f 0.1–0.5) which stained brown with aqueous potassium permanganate solution. Sodium chloride (4.7g, 77.6mmol) was added and the solution was stirred for a further 90min. The acetone was then evaporated and the aqueous phase extracted with chloroform (2 x 150cm^3, 1 x 50cm^3). The combined extracts were washed with 5% thiourea solution (50cm^3) and water (50cm^3), dried ($MgSO_4$), filtered and concentrated. The pale yellow oil thus obtained was subjected to flash chromatography (eluent hexane : ethyl acetate 7:3) to give (**147**) as a pale yellow oil (205mg, 5%); R_f 0.47 (ethyl acetate : hexane 3:2); $[\alpha]_D$ –33 (*c* 1.4, $CHCl_3$); δ_H ($CDCl_3$; 270MHz) 2.50–3.50 (1 H, br s, exch. D_2O, OH), 2.76 (1 H, ABX, $^2J_{AB}$ 14.5, $^3J_{5\text{-H, 6-Hax}}$ 11.2, 6-H_{ax}), 2.96 (1 H, ABX, $^2J_{AB}$ 14.5, $^3J_{5\text{-H, 6-Heq}}$ 5.2, 6-H_{eq}), 3.92–4.10 (2 H, m, 4-H, 5-H), 4.74, 4.85 (2 H, AB, J_{AB} 11.2, PhCH_2O), 5.71–5.80 (2 H, m, 2-H, 3-H), 7.21–7.54 (11 H, m, aromatic CH), 7.97–8.03 (4 H, m, 2-H and 6-H of benzoyl rings); δ_C ($CDCl_3$; 67.8 MHz) 43.96 (6-C), 68.16, 71.96 (2 x CH),

75.33 (Ph*C*H_2O), 77.58, 83.05 (2 x CH), 128.11, 128.38, 128.44, 128.57, 128.62 (aromatic CH), 129.10 (1-C of benzoyl ring[s]), 129.75, 130.00 (aromatic CH), 133.52, 133.85 (2 x 4-C of benzoyl rings), 137.27 (1-C of benzyl ring), 165.31, 165.49 (2 x Ph*C*O_2), 196.67 (1-C); *m/z* (FAB$^+$) 461 [(M+1)$^+$, 5%].

Further elution gave (**148**) (2.2g, 50%); R_f 0.42 (ethyl acetate : hexane 3:2); m.p. 149–150°C (ethanol); $[\alpha]_D$ –61.5 (*c* 4.1, $CHCl_3$); (Found: C, 70.2; H, 5.19. Calc. for $C_{27}H_{24}O_7$: C, 70.41; H, 5.26%); δ_H ($CDCl_3$; 270MHz) 2.68 (1 H, ABX, $^2J_{AB}$ 12, $^3J_{5\text{-H, 6-Hax}}$ 3.7, 6-H_{ax}), 2.86 (1 H, ABX, $^2J_{AB}$ 12, $^3J_{5\text{-H, 6-Heq}}$ 3.7, 6-H_{eq}), 3.17 (1 H, br s, exch. D_2O, OH), 4.09 (1 H, dd, *J* 2.5, 10, 4-H), 4.45 (1 H, br m, 5-H), 4.60, 4.74 (2 H, AB, J_{AB} 12, PhCH_2O), 5.67 (1 H, d, *J* 10, 2-H), 6.12 (1 H, t, *J* 10, 3-H), 7.15–7.58 (11 H, m, aromatic CH), 7.95–8.05 (4 H, m, 2-H and 6-H of benzoyl rings); δ_C ($CDCl_3$; 67.8 MHz) 42.38 (6-C), 58.27, 65.81, 71.75 (3 x CH), 72.39 (Ph*C*H_2O), 79.06 (4-C), 127.94, 128.10, 128.30, 128.35, 128.46 (aromatic CH), 128.75, 129.37 (2 x 1-C of benzoyl rings),129.71, 129.95 (aromatic CH), 133.21, 133.31 (2 x 4-C of benzoyl rings), 136.76 (1-C of benzyl ring), 165.27 , 165.53 (2 x Ph*C*O_2), 197.46 (1-C); *m/z* (FAB$^+$) 461 [(M+1)$^+$, 20%].

b) By catalytic Ferrier reaction using mercury (II) trifluoroacetate. A solution of (**146**) (14.5g, 30.6mmol) in acetone:water (5:2) containing 1% acetic acid (300cm^3) was stirred with mercury (II) trifluoroacetate (1.3g, 3.1mmol) for 30 h. The acetone was evaporated and the residual gum was extracted with $CHCl_3$ (3 x 200cm^3). The combined organic extracts were washed with water (200cm^3) and saturated aqueous NaCl solution (200cm^3), dried ($MgSO_4$), filtered and concentrated. Flash chromatography as above gave (**147**) (1.1g, 8%) and (**148**) (10.0g, 71%).

c) By Ferrier rearrangement using palladium (II) chloride. A solution of (**146**) (1.0g, 2.2mmol) in dioxane-5mM aqueous H_2SO_4 (2:1) (30cm^3) containing palladium (II) chloride (50mg, 0.3mmol) was heated under reflux for 1 h, then cooled. The solvents were evaporated. Purification and flash chromatography as in method *b)* gave (**147**) (61mg, 6%) and (**148**) (698mg, 72%).

6.2.13 (2*S*, 3*R*, 4*S*)-2,3-Dibenzoyloxy-4-benzyloxycyclohex-5-enone ethylene acetal (169) and (2*S*, 3*R*, 4*S*, 5*S*)-2,3-dibenzoyloxy-4-benzyloxy-5-hydroxycyclohexanone ethylene acetal (168)

The method employed is a modification of that described by Ferrier and Haines.[173] A mixture of (**148**) (5.2g, 11.3mmol), ethylene glycol (7.5cm^3), dioxane (50cm^3), toluene (75cm^3) and conc. H_2SO_4 (0.5cm^3) was heated under reflux for 3.5 h with continuous azeotropic removal of water (Dean-Stark trap). TLC (ethyl acetate) indicated consumption of starting material (R_f 0.62) to give two products (R_f 0.79 and 0.48). Pyridine (2cm^3) was added, the solution was cooled and the solvents evaporated. The dark brown residue thus obtained was extracted with ether (250cm^3) and the organic solution was washed with 5M aqueous HCl (150cm^3) and water (2 x 150cm^3), dried ($MgSO_4$), filtered and concentrated to give a pale yellow oil. Flash chromatography (eluent hexane : ethyl acetate 1:1) gave (**169**) (1.8g, 33%); m.p. 125–127°C (ethanol); $[\alpha]_D$ +14.8 (*c* 1.6, $CHCl_3$); (Found: C, 71.8; H, 5.37. Calc. for $C_{29}H_{26}O_7$: C, 71.58; H, 5.39%); δ_H ($CDCl_3$; 400MHz) 3.89–4.12 (4 H, m, OCH_2CH_2O), 4.45 (1 H, dt, *J* 8.3, 1.5, 4-H), 4.57, 4.69 (2 H, AB, J_{AB} 11.9, $PhCH_2O$), 5.63 (1 H, d, *J* 11.2, 2-H), 5.75 (1 H, dd, $^4J_{4\text{-H},6\text{-H}}$ 1.5, $^3J_{5\text{-H},6\text{-H}}$ 10.3, 6-H), 5.97–6.01 (2 H, m, 3-H, 5-H), 7.19–7.46 (11 H, m, aromatic CH), 7.86–7.96 (4 H, m, 2-H and 6-H of benzoyl rings); δ_C ($CDCl_3$; 67.8MHz) 66.15, 66.51 (OCH_2CH_2O), 70.84 ($PhCH_2O$), 72.75, 72.85, 76.56 (2-C, 3-C, 4-C), 106.02 (1-C), 127.73, 127.89, 128.18, 128.35, 128.98 (5-C, 6-C, aromatic CH), 129.24 (1-C of benzoyl ring[s]), 129.69, 129.77 (aromatic CH), 132.92, 133.63 (2 x 4-C of benzoyl rings), 137.67 (1-C of benzyl ring), 165.60 ($PhCO_2$); *m/z* (FAB$^+$) 487 [(M+1)$^+$, 5%].

Further elution gave (**168**) (3.1g, 54%); m.p. 129–131°C (ethanol); $[\alpha]_D$ –57.4 (*c* 0.6, $CHCl_3$); (Found: C, 68.7; H, 5.78. Calc. for $C_{29}H_{28}O_8$: C, 69.02; H, 5.60%); δ_H ($CDCl_3$; 270MHz) 1.96 (1 H, ABX, $^2J_{AB}$ 15, ^3J 3.3, 6-H_{ax}), 2.25 (1 H, ABX, $^2J_{AB}$ 15, ^3J 3.8, 6-H_{eq}), 3.24 (1 H, d, *J* 6.6, exch. D_2O, OH), 3.71 (1 H, dd, *J* 3.3, 9.5, 4-H), 3.80–3.92 (2 H, m, OCH_2CH_2O), 4.06–4.16 (2 H, m, OCH_2CH_2O), 4.21 (1 H, br m, 5-H), 4.60, 4.71 (2 H, AB, J_{AB} 12, $PhCH_2O$), 5.48 (1 H, d, *J* 9.5, 2-H), 6.01 (1 H, t, *J* 9.5, 3-H), 7.16–7.52 (11 H, m, aromatic CH), 7.88–7.97 (4 H, m, 2-H and 6-H of benzoyl rings); δ_C

($CDCl_3$; 67.8 MHz) 36.63 (6-C), 65.40 (OCH_2CH_2O), 65.55 (CH), 66.72 (OCH_2CH_2O), 70.98 (CH), 71.88 ($PhCH_2O$), 73.85, 78.93 (2 x CH), 108.27 (1-C), 127.79 (1-C of benzoyl ring), 127.83, 128.18, 128.30, 128.35 (aromatic CH), 129.19 (1-C of benzoyl ring[s]), 129.66, 129.72 (aromatic CH), 132.87, 133.16 (2 x 4-C of benzoyl rings), 137.31 (1-C of benzyl ring), 165.42, 165.48 (2 x $PhCO_2$); *m/z* (FAB^+) 505 [$(M+1)^+$, 10%].

6.2.14 Attempted Mitsunobu Reaction on (168)

To a solution of (**168**) (480mg, 1.0mmol) and benzoic acid (174mg, 1.5mmol, dried *in vacuo* over P_2O_5 at 45°C for 18 h) in freshly distilled, dry THF ($5cm^3$) at 0°C under an atmosphere of nitrogen was added tri-*n*-butylphosphine ($0.35cm^3$, 1.5mmol). After 2 min 1,1'-(azodicarbonyl)-dipiperidine (360mg, 1.5mmol) was added. The system was allowed to warm to room temperature and was stirred for 24 h. The system was diluted with ether ($100cm^3$) and washed with water ($30cm^3$), dried ($MgSO_4$), filtered and concentrated. The residue was subjected to flash chromatography (eluent hexane : ethyl acetate 1:1) to give exclusively starting material (422mg, 88%).

6.2.15 (2*S*, 3*R*, 4*S*, 5*R*)-5-Acetoxy-2,3-dibenzoyloxy-4-benzyloxycyclohexanone ethylene acetal (170)

a) By inversion of triflate. The method employed is a modification of that described by Lampe.[66] To a solution of (**168**) (84mg, 0.2mmol) in dry CH_2Cl_2-dry pyridine (5:1) ($3cm^3$) at –78°C under an atmosphere of nitrogen was added triflic anhydride ($0.03cm^3$) dropwise. The solution was allowed to warm to room temperature and was stirred for 30 min, when TLC (ethyl acetate : hexane 3:2) indicated consumption of starting material (R_f 0.16) to give a product (R_f 0.56). The solvents were evaporated. To the orange residue were added caesium acetate (200mg, 1mmol, dried *in vacuo* over P_2O_5 at 60°C for 24 h), and dry DMF ($3cm^3$). This mixture was stirred at room temperature for 3 h when TLC (ether) indicated presence of a major product (R_f 0.60) and a minor product (R_f 0.53). The solvent was evaporated and the orange residue was extracted with ethyl acetate ($50cm^3$). The organic extract was washed with saturated aqueous

NaCl solution (30cm^3) and water (30cm^3), dried ($MgSO_4$), filtered and concentrated. Flash chromatography (eluent hexane : ethyl acetate 7:3) gave (**169**) (43mg, 53%).

Further elution gave (**170**) (11mg, 12%); m.p. 217–222°C (ethyl acetate-hexane); $[\alpha]_D$ -32.2 (*c* 2.1, $CHCl_3$); (Found: C, 68.2; H, 5.44. Calc. for $C_{31}H_{30}O_9$: C, 68.11; H, 5.54%); δ_H ($CDCl_3$; 270MHz) 1.84 (1 H, t, $^2J = {}^3J$ = 12.5, 6-H_{ax}), 2.01 (3 H, s, $COCH_3$), 2.30 (1 H, dd, ^2J 12.5, ^3J 5.1, 6-H_{eq}), 3.82–3.92 (3 H, m, OCH_2CH_2O, 4-H), 4.07–4.12 (2 H, m, OCH_2CH_2O), 4.63, 4.63 (2 H, AB, J_{AB} 11.6, $PhCH_2O$), 5.23 (1 H, ddd, *J* 12.5, 11.4, 5.1, 5-H), 5.55 (1 H, d, *J* 10, 2-H), 5.81 (1 H, t, *J* 10, 3-H), 7.10–7.56 (11 H, m, aromatic CH), 7.86–8.04 (4 H, m, 2-H and 6-H of benzoyl rings); δ_C ($CDCl_3$; 100MHz) 21.03 (CH_3CO_2), 37.03 (6-C), 65.84, 66.53 (OCH_2CH_2O), 71.14, 72.62, 74.05 (2-C, 3-C, 5-C), 74.56 ($PhCH_2O$), 80.82 (4-C), 106.07 (1-C), 127.63, 127.87, 128.22, 128.25, 128.40, 129.17, 129.55 (aromatic CH), 129.66, 129.77 (2 x 1-C of benzoyl rings), 132.97, 133.23 (2 x 4-C of benzoyl rings), 137.71 (1-C of benzyl ring), 165.36, 165.58 (2 x $PhCO_2$), 169.84 (CH_3CO_2); *m/z* (FAB^+) 547 [$(M+1)^+$, 15%].

b) By inversion of mesylate. The method employed is a modification of that described by Torisawa *et al.*[238] A solution of (**168**) (848mg, 1.7mmol) in dry dichloromethane-dry pyridine (2:1) (9cm^3) was treated with mesyl chloride (0.15cm^3, 1.9mmol) at room temperature for 5 h, when TLC (ethyl acetate : hexane 3:2) indicated conversion of starting material to a product (R_f 0.58). The solvents were evaporated and co-evaporated with toluene (3 x 50cm^3). The residue was dissolved in ethyl acetate (150cm^3) and this solution was washed with water (50cm^3) to remove salts, then dried ($MgSO_4$), filtered and concentrated to give a pale yellow solid. Dry toluene (40cm^3), caesium acetate (3.2g) and 18-crown-6 (444mg, 1.7mmol) were added and this mixture was heated under reflux for 4 days. Purification and flash chromatography as in method *a)* gave (**169**) (441mg, 54%) and (**170**) (167mg, 18%).

6.2.16 (6*S*)-6-Benzoyloxy-4-benzyloxycyclohex-4-ene-1,3-dione 1-ethylene acetal (172)

Oxalyl chloride (1.7cm^3 of a 2M solution in dichloromethane, 3.4mmol) was added to a 100cm^3 three-necked flask at -50 – -60°C under an atmosphere of nitrogen. Dry DMSO (0.44cm^3, 6.2mmol) was added dropwise over 5 min (*copious evolution of gas!*). After 5 min, a solution of (**168**) (1.6g, 3.1mmol) in dry dichloromethane was added dropwise. The system was stirred at -50 – -60°C for 20min, when triethylamine (1.7cm^3, 12.5mmol) was added dropwise. The mixture was allowed to warm to room temperature over 20 min, then the solvents were evaporated. The residue was extracted with dichloromethane (150cm^3) and the organic extract was washed with water (150cm^3), dried ($MgSO_4$), filtered and concentrated. The yellow oil thus obtained was purified by flash chromatography (eluent hexane : ethyl acetate 7:3) to give the title compound (1.2g, 99%) which crystallised as fine white needles from ethanol; R_f 0.59 (ethyl acetate : hexane 3:2); m.p. 96–97°C; $[\alpha]_D$ +125.3 (*c* 2.2, $CHCl_3$); (Found: C, 69.4; H, 5.28. Calc. for $C_{22}H_{20}O_6$: C, 69.45; H, 5.30%); δ_H ($CDCl_3$; 400MHz) 2.81 (1 H, ABX, $^2J_{AB}$ 16.1, ^4J 1.0, 2-H$_{ax}$), 3.19 (1 H, AB, $^2J_{AB}$ 16.1, 2-H$_{eq}$), 3.96–4.11 (4 H, m, OCH_2CH_2O), 4.87 (2 H, s, $PhCH_2O$), 5.84 (1 H, ABX, $^3J_{AB}$ 5.4, ^4J 1.0, 6-H), 5.90 (1 H, AB, $^3J_{AB}$ 5.4, 5-H), 7.26–7.62 (8 H, m, aromatic CH), 8.01–8.06 (2 H, m, 2-H and 6-H of benzoyl ring); δ_C ($CDCl_3$; 100MHz) 46.05 (2-C), 65.73, 65.82 (OCH_2CH_2O), 69.95 (6-C), 70.21 ($PhCH_2O$), 107.13 (1-C), 110.99 (5-C), 127.63, 128.24, 128.35, 128.55, 128.62, 128.80 (aromatic CH), 129.55 (1-C of benzoyl ring), 129.70 (aromatic CH), 133.47 (4-C of benzoyl ring), 135.24 (1-C of benzyl ring), 152.50 (4-C), 165.78 ($PhCO_2$), 189.85 (3-C); *m/z* (FAB$^+$) 381 [(M+1)$^+$, 10%].

6.2.17 (4*S*, 5*R*, 6*R*)-4,5-Dibenzoyloxy-6-benzyloxycyclohexan-1,3-dione 3-ethylene acetal (171)

A mixture of (**168**) (1.0g, 2.0mmol), pyridinium chlorochromate (5.0g, 23.2mmol), powdered molecular sieves and dry dichloromethane (15cm^3) was stirred at room temperature for 6 h, when TLC (ethyl acetate) indicated conversion of starting material (R_f 0.42) to a product (R_f 0.67). Ether (250cm^3) was added to the dark brown suspension and the mixture was filtered through Celite. The filtrate was washed with

water (200cm^3) and saturated aqueous NaCl solution (200cm^3), dried ($MgSO_4$), filtered and concentrated. Crystallisation from ethanol gave the title compound (526mg) and further quantities (total 620mg, 62%) were isolated by flash chromatography of the mother liquors (eluent hexane : ethyl acetate 1:1); m.p. 185–187°C; $[\alpha]_D$ –34.0 (*c* 1.9, $CHCl_3$); (Found: C, 69.1; H, 5.22. Calc. for $C_{29}H_{26}O_8$: C, 69.30; H, 5.22%); δ_H ($CDCl_3$; 400MHz) 2.77, 2.95 (2 H, AB, J_{AB} 14.5, 2-H, 2-H'), 3.84–3.94 (2 H, m, OCH_2CH_2O), 4.03–4.12 (2 H, m, OCH_2CH_2O), 4.31 (1 H, m, 6-H), 4.55 (1 H, d, *J* 12.2, PhC*H*HO), 4.93 (1 H, d, *J* 12.7, PhCH*H*O), 5.88–5.90 (2 H, m, 4-H, 5-H), 7.08–7.52 (11 H, m, aromatic CH), 7.87–7.96 (4 H, m, 2-H and 6-H of benzoyl rings); δ_C ($CDCl_3$; 100MHz) 48.10 (2-C), 66.22, 66.48 (OCH_2CH_2O), 71.50 (CH), 72.65 ($PhCH_2O$), 73.85 (CH), 81.98 (6-C), 105.77 (3-C), 127.79, 128.00, 128.22, 128.43 (aromatic CH), 128.86, 129.27 (2 x 1-C of benzoyl rings), 129.74, 133.06, 133.36 (2 x 4-C of benzoyl rings), 136.86 (1-C of benzyl rings), 165.13, 165.34 (2 x $PhCO_2$), 199.69 (1-C); *m/z*: (FAB^+) 503 [$(M+1)^+$, 90%].

6.2.18 Reduction of (171) with (*R*)-Alpine-Hydride

To a solution of (**171**) (150mg, 0.3mmol) in THF (5cm^3) at –78°C was added (*R*)-Alpine-Hydride (1.2cm^3 of a 0.5M solution in THF, 0.6mmol) and the system was kept at –78°C for 1 h. After dropwise addition of 1M aqueous HCl (0.5cm^3), the mixture was evaporated to dryness. The residue was extracted with chloroform (50cm^3) and the organic extract was washed with water (50cm^3), dried ($MgSO_4$), filtered and concentrated. Flash chromatography (eluent hexane : ethyl acetate 2:3) gave exclusively (**168**) (129mg, 88%); m.p. and m.m.p. 128–130°C.

6.2.19 D-1,6-Di-*O*-benzoyl-5-*O*-benzyl-3-deoxy-*myo*-inositol (176) and D-1,2-di-*O*-benzoyl-3-*O*-benzyl-5-deoxy-*scyllo*-inositol (177)

To a solution of (**147**) (533mg, 1.2mmol) in dioxane (15cm^3) was added sodium borohydride (132mg, 3.5mmol). This mixture was stirred at room temperature for 1 h, when TLC (ethyl acetate) indicated consumption of starting material (R_f 0.65) to give a minor product (R_f 0.52) and a major product (R_f 0.44). After dropwise addition of 1M aqueous HCl (0.5cm^3), the system was evaporated to dryness. The residue was

extracted with chloroform (2 x 50cm^3) and the combined organic extracts were washed with water (50cm^3), dried ($MgSO_4$), filtered and concentrated. The residue was subjected to flash chromatography (eluent hexane : ethyl acetate 1:1) to give (**176**) as a waxy solid (115mg, 22%); $[\alpha]_D$ –61.1 (*c* 2.0, $CHCl_3$); δ_H ($CDCl_3$; 400MHz) 1.69 (1 H, ddd, ^2J 14.0, ^3J 11.9, 2.4, 3-H_{ax}), 2.34 (1 H, dt, ^2J 14.0, ^3J 4.3, 3-H_{eq}), 2.43, 2.53 (2 H, 2 br s, exch. D_2O, 2 x OH), 3.62 (1 H, t, *J* 9.5, 5-H), 4.24 (1 H, ddd, *J* 4.9, 9.2, 11.9, 4-H), 4.36 (1 H, br m, 2-H), 4.66, 4.75 (2 H, AB, J_{AB} 11.3, PhCH_2O), 5.26 (1 H, dd, *J* 2.8, 10.1, 1-H), 5.99 (1 H, t, *J* 9.8, 6-H), 7.16–7.50 (11 H, m, aromatic CH), 7.91–8.07 (4 H, m, 2-H and 6-H of benzoyl rings); *m/z* (FAB$^+$) 463 [(M+1)$^+$, 80%].

Further elution gave (**177**) (376mg, 70%); m.p. 82–87°C (ethyl acetate-hexane); $[\alpha]_D$ –65.0 (*c* 1.4, $CHCl_3$); (Found: C, 70.1; H, 5.68. Calc. for $C_{27}H_{26}O_7$:C, 70.10; H, 5.67%); δ_H ($CDCl_3$; 400MHz) 1.65 (1 H, q, $^2J = {}^3J$ =11.9, 5-H_{ax}), 2.34 (1 H, dt, ^2J 12.8, ^3J 4.6, 5-H_{eq}), 2.51, 2.60 (2 H, 2 br s, exch. D_2O, 2 x OH), 3.58 (1 H, t, *J* 9.3, 3-H), 3.71 (1 H, br m, simplifies to ddd on D_2O exch., *J* 4.6, 9.2, 11.6, 4-H), 3.85 (1 H, br m, simplifies to ddd on D_2O exch., *J* 4.6, 9.5, 11.8, 6-H), 4.53, 4.64 (2 H, AB, J_{AB} 11.2, PhCH_2O), 5.25 (1 H, t, *J* 9.8, 1-H), 5.47 (1 H, t, *J* 9.8, 2-H), 7.06–7.43 (11 H, m, aromatic CH), 7.82–7.87 (4 H, m, 2-H and 6-H of benzoyl rings); δ_C ($CDCl_3$; 100 MHz) 36.66 (5-C), 68.15, 68.44, 72.94 (3 x CH), 75.19 (PhCH_2), 77.55, 83.77 (2 x CH), 128.02, 128.33, 128.39, 128.54 (aromatic CH), 128.99, 129.29 (2 x 1-C of benzoyl rings), 129.62, 129.82 (aromatic CH), 133.22, 133.33 (2 x 4-C of benzoyl rings), 137.50 (1-C of benzyl ring), 165.66, 166.96 (2 x PhCO_2); *m/z* (FAB$^+$) 463 [(M+1)$^+$, 30%].

6.2.20 D-1-*O*-benzyl-3-deoxy-*scyllo*-inositol (138)

To a solution of (**177**) (370mg, 0.8mmol) in methanol (25cm^3) was added sodium hydroxide (128mg, 3.2mmol) and this mixture was heated under reflux for 1 h, when TLC (ethyl acetate : methanol 4:1) indicated conversion of starting material (R_f 0.82) to a product (R_f 0.43). The solution was cooled and concentrated. The residue was purified by flash chromatography (eluent ethyl acetate : methanol 9:1) to give the title compound as a white solid (174mg, 86%); m.p. 119–121°C; $[\alpha]_D$ –3.3 (*c* 1.2, $CHCl_3$); δ_H (D_2O; ref. int. D_2O; 400MHz) 1.47 (1 H, q, $^2J = {}^3J_{3\text{-Hax},2\text{-H}} = {}^3J_{3\text{-Hax},4\text{-H}}$ = 12.2, 3-H_{ax}), 2.17 (1 H, dt, ^2J 12.2, ^3J 4.6, 3-H_{eq}), 3.22–3.34 (3 H, m, 1-H, 5-H, 6-H), 3.50 (1 H, ddd,

J 4.6, 9.7, 12.2, 2-H or 4-H), 3.63 (1 H, br m, 2-H or 4-H), 4.82 (2 H, s, PhCH_2O), 7.35–7.65 (5 H, m, aromatic CH); δ_C (D_2O; 67.8 MHz) 35.66 (3-C), 67.07, 67.14, 72.63 (inositol CH), 73.70 (Ph*C*H_2O), 75.68, 84.02 (inositol CH), 127.14, 127.47, 127.64, 128.31 (aromatic CH), 136.43 (1-C of benzyl ring); *m/z* (FAB^+) 255 [$(M+1)^+$, 10%], (FAB^-) 253 [$(M-1)^-$, 30%], 407 [$(M+NBA)^-$, 30%].

6.2.21 D-4-*O*-Benzyl-1,2,3,5-tetrakis(di-*p*-chlorobenzylphospho)-6-deoxy-*scyllo*-inositol (178)

A mixture of 1*H*-tetrazole (139mg, 1.98mmol), dry dichloromethane ($5cm^3$) and bis(*p*-chlorobenzyloxy)(diisopropylamino)phosphine[242] (546mg, 1.32mmol) was stirred at room temperature for 20 min, whereupon (**138**) (42mg, 0.17mmol) was added and stirring was continued for a further 30 min. TLC (ethyl acetate) indicated conversion of starting material (R_f 0.04) to a product (R_f 0.71–0.83). The mixture was cooled to –78°C and *m*-chloroperoxybenzoic acid (900mg) was added. The solution was allowed to warm to room temperature and after 10 min TLC (ethyl acetate : hexane 3:2) showed a new product (R_f 0.10). The solution was diluted with ether ($100cm^3$) and the organic extract was washed with $50cm^3$ each of 10%$^w/_v$ $Na_2S_2O_3$ solution, 1M aqueous HCl, saturated aqueous $NaHCO_3$ solution and saturated aqueous NaCl solution. The organic solution was dried ($MgSO_4$), filtered and concentrated. Purification of the residue by flash chromatography (eluent hexane : ethyl acetate 7:3 then 1:1) gave the title compound as a waxy solid (140mg, 54%); $[\alpha]_D$ –4.5 (*c* 5.3, $CHCl_3$); (Found: C, 52.9; H, 3.95. Calc. for $C_{69}H_{62}O_{17}P_4Cl_8$:C, 52.87; H, 3.99%); δ_H ($CDCl_3$; 400MHz) 1.81 (1 H, q, $^2J = {}^3J$ = 11.9, 6-H_{ax}), 2.88 (1 H, ddd, ^2J 11.9, ^3J 4.6, 4.8, 6-H_{eq}), 3.61 (1 H, t, *J* 8.9, 4-H), 4.29–4.61 (4 H, m, 1-H, 2-H, 3-H, 5-H), 4.70–5.01 (18 H, m, 9 x ArCH_2O AB systems), 6.85–7.30 (37 H, m, aromatic CH); δ_C ($CDCl_3$; 100MHz) 34.77 (6-C), 69.45, 69.50, 69.54, 69.58, 69.63, 69.70, 69.76, 69.85 (8 x Ar*C*H_2O), 73.40, 75.10 (2 x CH), 75.41 (Ph*C*H_2O), 78.10, 79.73, 81.94 (3 x CH), 127.15, 127.73, 128.39, 129.12, 129.45, 129.52, 129.60, 129.69, 129.76, 129.94, 129.98, 130.02, 130.07, 130.16, 130.16 (aromatic CH), 134.53, 134.61, 134.66, 134.74, 134.77, 134.86, 134.90, 134.94, 135.19, 135.27, 135.34, 135.39, 135.50, 135.54, 135.63 (1-C and 4-C of 8 x *p*-chlorobenzylphospho rings), 138.50 (1-C of benzyl ring); δ_P ($CDCl_3$; 162MHz) –2.25,

–1.78, –1.73, –1.70 (4 s); *m/z*: (FAB$^+$) 1567 (20%), 1569 (50%), 1571 (68%), 1573 (45%), 1575 (18%), 1577 (6%) [all $(M+1)^+$].

6.2.22 D-2-Deoxy-*myo*-inositol-1,3,4,5-tetrakisphosphate (78)

Ammonia was condensed into a three-necked flask at –78°C. An excess of sodium was added to dry the ammonia, about 30cm^3 of which was then distilled into a second three-necked flask and kept at –78°C. Sodium was added until the solution remained blue-black for 10 min. A solution of (**178**) (71mg, 45μmol) in dry dioxane (1.5cm^3) was added and the mixture was stirred for 2 min. The reaction was quenched with methanol (1cm^3), followed by water (1cm^3). The solvents were evaporated and the residue dissolved in de-ionised water (400cm^3) and purified by ion-exchange chromatography on Q Sepharose fast flow, eluting with a gradient of triethylammonium bicarbonate buffer (0–1M) pH 9.0. The triethylammonium salt of the title compound eluted between 250–280mM buffer; $[\alpha]_D$ 0.0 (*c* 0.3, calc. for free acid, TEAB, pH 7.5); δ_H (D_2O; pH *ca.* 6, 400MHz) 1.55 (1 H, q, $^2J = {}^3J$ = 11.6, 2-H_{ax}), 2.37 (1 H, br m, 2-H_{eq}), 3.44 (1 H, t, *J* 9.2, 6-H), 3.84–3.95 (4 H, m, 1-H, 3-H, 4-H, 5-H); δ_P (D_2O; pH *ca.* 6, 400MHz) (^{1}H-coupled) –0.27 (d, J_{HP} 7.7), 0.05 (d, J_{HP} 8.2), 0.72 (d, J_{HP} 8.2), 0.83 (d, J_{HP} 9.0); *m/z*: (FAB$^-$) 483 [$(M-1)^-$, 100%]; (Found: M^- 482.926. Calc. for $C_6H_{16}O_{17}P_4$ $[M-H]^-$: 482.926).

6.3 Synthesis of (1*R*, 2*R*, 3*S*, 4*R*, 5*S*)-3-Hydroxy-5-hydroxymethyl-1,2,4-trisphosphocyclopentane

6.3.1 *p*-Methoxybenzaldehyde dimethyl acetal (187)

The method employed is a modification of that described by Johansson and Samuelsson.[252] A 2 litre flask containing a stirring solution of *p*-methoxybenzaldehyde (272cm^3, 304g, 2.24mol), trimethyl orthoformate (274cm^3, 266g, 2.51mol) and PTSA (2.4g) was fitted with an air condenser, attached to a water pump *via* a three-way tap and evacuated. After 6 h the vacuum was broken, sodium carbonate (2.7g, 25mmol) was added and the suspension was stirred at ambient pressure for 1 h, then filtered. Distillation of the filtrate *in vacuo* gave the title compound as a colourless liquid (338g, 83%); b.p. *ca.* 110°C at 0.2mbar [lit.[252] *ca.* 100°C at 0.2mbar]; *d* 1.06gcm^{-3} [lit.[252] 1.06gcm^{-3}]; δ_H 3.31 (6 H, s, CH[OCH_3]$_2$), 3.81 (3 H, s, ArOCH_3), 5.35 (1 H, s, C*H*[OCH$_3$]$_2$), 6.89 (2 H, d, *J* 8.6, 3-H, 5-H), 7.36 (2 H, d, *J* 8.6, 2-H, 6-H).

6.3.2 Methyl 4,6-*O*-(*p*-methoxybenzylidene)-α-D-glucopyranoside (186)

A 1 litre flask containing methyl α-D-glucopyranoside (103g, 0.53mol), *p*-methoxybenzaldehyde dimethyl acetal (96cm^3, 0.56mol), PTSA (2g) and DMF (500cm^3) was fitted with an air condenser, attached to a water pump *via* a three-way tap and evacuated. The system was stirred at 70°C until methanol ceased to condense (4 h). The solution was cooled and concentrated to give a white residue. Crystallisation from 2%$^{w}/_{v}$ aqueous NaHCO$_3$ solution gave the title compound as fine white needles (134g, 81%); m.p. 202°C [lit.[252] 194°C]; $[\alpha]_D$ +88.4 (*c* 1.2, DMF) [lit.[252] +97.4].

6.3.3 Methyl 2-*O*-benzyl-4,6-*O*-(*p*-methoxybenzylidene)-α-D-glucopyranoside (188) and methyl 3-*O*-benzyl-4,6-*O*-(*p*-methoxybenzylidene)-α-D-glucopyranoside (189)

A mixture of (**186**) (15.5g, 49.7mmol), dibutyltin oxide (13.6g, 54.6mmol) and tetrabutylammonium iodide (18.4g, 49.7mmol) was heated under reflux for 2 h in acetonitrile (600cm^3) *via* a Soxhlet thimble containing 4Å molecular sieves. The

solution was cooled to room temperature and benzyl bromide (6.5cm^3, 54.6mmol) was added. The system was heated under reflux for a further 16 h, then cooled. Triethylamine (25cm^3) was added and stirring was continued for 3 h. The solvents were evaporated and the orange residue was triturated with ether (500cm^3). The ethereal extract was vigorously stirred with saturated aqueous $NaHCO_3$ solution (200cm^3) for 2 h, and the resulting suspension was filtered through Celite. The organic layer was collected, washed with water (200cm^3) dried ($MgSO_4$), filtered and concentrated. The white residue thus obtained was subjected to flash chromatography (eluent hexane : ethyl acetate 7:3) to give (**188**) (9.6g, 48%); R_f 0.53 (hexane : ethyl acetate 2:3); m.p. 135–136.5°C (from ethanol); $[\alpha]_D$ +31.9 (*c* 1.3, $CHCl_3$); (Found: C, 65.4; H, 6.47. Calc. for $C_{22}H_{26}O_7$: C, 65.64; H, 6.52%); δ_H ($CDCl_3$; 400MHz) 2.65 (1 H, d, *J* 2.0, exch. D_2O, OH), 3.37 (3 H, s, OCH_3), 3.44–3.49 (2 H, m, 2-H, 4-H), 3.68 (1 H, t, ^2J=^3J=10, 6-H_{ax}), 3.78 (3 H, s, $ArOCH_3$), 3.77–3.80 (1 H, m, 5-H), 4.13 (1 H, td, *J* 9.7, 2.0, simplifies to t on D_2O exch., 3-H), 4.23 (1 H, dd, ^2J 10, ^3J 4.6, 6-H_{eq}), 4.60 (1 H, d, *J* 3.4, 1-H), 4.69, 4.78 (2 H, AB, J_{AB} 12.2, $C_6H_5CH_2O$), 5.47 (1 H, s, 7-H), 6.87 (2 H, d, *J* 8.8, 3-H and 5-H of *p*-methoxyphenyl ring), 7.25–7.42 (7 H, m, aromatic CH); δ_C ($CDCl_3$; 100.4 MHz) 55.26, 55.31 (2 x OCH_3), 61.97 (CH), 68.88 (6-C), 70.23 (CH), 73.20 ($C_6H_5CH_2O$), 73.31 (CH), 79.49 (2-C), 98.53 (1-C), 101.89 (7-C), 113.59 (3-C and 5-C of *p*-methoxyphenyl ring), 127.56, 127.64, 128.04, 128.10, 128.52 (aromatic CH), 129.56 (1-C of *p*-methoxyphenyl ring), 137.88 (1-C of benzyl ring), 160.14 (4-C of *p*-methoxyphenyl ring); *m/z* (FAB^+) 403 [$(M+1)^+$, 40%].

Further elution gave (**189**) (2.2g, 11%); R_f 0.35 (hexane : ethyl acetate 2:3); m.p. 176–178°C (from ethanol); $[\alpha]_D$ +76.2 (*c* 1.7, $CHCl_3$); (Found: C, 65.5; H, 6.48. Calc. for $C_{22}H_{26}O_7$: C, 65.64; H, 6.52%); δ_H ($CDCl_3$; 270 MHz) 2.42 (1 H, d, *J* 7.3, exch. D_2O, OH), 3.43 (3 H, s, OCH_3), 3.58–3.84 (8 H, m, 2-H, 3-H, 4-H, 5-H, 6-H_{ax}, $ArOCH_3$), 4.34 (1 H, dd, ^3J 3.8, ^2J 9.3, 6-H_{eq}), 4.75–4.96 (3 H, m, AB, J_{AB} 11.5, $C_6H_5CH_2O$ overlapping with 1-H), 5.51 (1 H, s, 7-H), 6.90 (2 H, d, *J* 8.8, 3-H and 5-H of *p*-methoxyphenyl ring), 7.24–7.42 (7 H, m, aromatic CH); δ_C ($CDCl_3$; 67.8 MHz) 55.20, 55.29 (2 x OCH_3), 62.53 (CH), 68.88 (6-C), 72.33 (CH), 74.67 ($C_6H_5CH_2O$), 78.78, 81.82 (CH), 99.82 (1-C), 101.20 (7-C), 113.51 (3-C and 5-C of *p*-methoxyphenyl ring), 127.28, 127.60, 127.89, 128.28 (aromatic CH), 129.81 (1-C of *p*-methoxyphenyl ring),

138.45 (1-C of benzyl ring), 159.95 (4-C of *p*-methoxyphenyl ring); *m/z* (FAB^+) 403 [$(M+1)^+$, 30%].

6.3.4 Methyl 2-*O*-benzyl-3-*O*-(*p*-methoxybenzyl)-4,6-*O*-(*p*-methoxybenzylidene)-α-D-glucopyranoside (190)

A solution of (**188**) (9.6g, 23.8mmol) in dry DMF (50cm^3) was sequentially treated with sodium hydride (857mg of an 80%$^{w}/_{w}$ dispersion in mineral oil, 28.6mmol) and *p*-methoxybenzyl chloride (3.4cm^3, 25.0mmol). The mixture was stirred at room temperature for 3 h. Methanol (10cm^3) was added and stirring was continued for 1 h. The solvents were evaporated and the residue was extracted with chloroform (3 x 150cm^3). The combined organic extracts were washed with water (2 x 100cm^3), dried ($MgSO_4$), filtered and concentrated to give crude (**190**) (11.8g). Crystallisation from ethanol gave pure (**190**) (10.9g, 87%); R_f 0.60 (hexane : ethyl acetate 2:3); m.p. 144–145°C; $[\alpha]_D$ –30.0 (*c* 1.2, $CHCl_3$); (Found: C, 68.9; H, 6.67. Calc. for $C_{30}H_{34}O_8$: C, 68.94; H, 6.56%); δ_H ($CDCl_3$;270 MHz) 3.40 (3 H, s, OCH_3), 3.50–3.59 (2 H, m, 2-H, 4-H), 3.64–3.98 (8 H, m, 5-H, 6-H_{ax}, 2 x $ArOCH_3$), 4.02 (1 H, t, *J* 9.1, 3-H), 4.39 (1 H, dd, *J* 4.3, 9.8, 6-H_{eq}), 4.57 (1 H, d, *J* 3.7, 1-H), 4.67–4.88 (4 H, m, 2 x $ArCH_2O$ AB systems), 5.50 (1 H, s, 7-H), 6.82–6.92 (4 H, m, 3-H and 5-H of *p*-substituted rings), 7.25–7.42 (9 H, m, aromatic CH); δ_C ($CDCl_3$; 67.8 MHz) 55.24, 55.27, 55.31 (3 x OCH_3), 62.34 (CH), 69.01 (6-C), 73.76, 74.99 (2 x $ArCH_2O$), 78.30, 79.18, 82.08 (CH), 99.27 (1-C), 101.23 (7-C), 113.56, 113.72 (2 x 3-C and 5-C of *p*-substituted rings), 127.32, 127.86, 128.07, 128.41, 129.66, 129.97 (aromatic CH), 130.92 (1-C of *p*-substituted ring[s]), 138.22 (1-C of benzyl ring), 159.18, 159.99 (2 x 4-C of *p*-substituted rings); *m/z* (FAB^+) 523 [$(M+1)^+$, 10%].

6.3.5 Methyl 2-*O*-benzyl-3,6-di-*O*-(*p*-methoxybenzyl)-α-D-glucopyranoside (194) and methyl 2-*O*-benzyl-3,4-di-*O*-(*p*-methoxybenzyl)-α-D-glucopyranoside (185)

a) The method employed is a modification of that described by Johansson and Samuelsson.[252] A solution, kept at 0°C, of trimethylsilyl chloride (12.5cm^3, 98.4mmol) in dry acetonitrile (15cm^3) was added dropwise under nitrogen to a vigorously stirred mixture of (**190**) (8.6g, 16.4mmol), sodium cyanoborohydride (6.2g, 98.4mmol), 3Å

molecular sieves and dry acetonitrile (150cm^3) at 0°C. The suspension was allowed to warm to room temperature and was stirred under nitrogen for 3.5 h, when TLC (hexane : ethyl acetate 2:3) indicated conversion of starting material (R_f 0.62) into a minor product (R_f 0.51) and a major product (R_f 0.28). The suspension was filtered through Celite into ice-cold saturated aqueous $NaHCO_3$ solution (300cm^3) and the residue was well washed with acetonitrile. The aqueous layer was extracted with ether (2 x 200cm^3) and the combined organic extracts were washed with saturated aqueous $NaHCO_3$ solution (250cm^3), dried ($MgSO_4$), filtered and concentrated. The yellow residue thus obtained was subjected to flash chromatography (eluent hexane : ethyl acetate 7:3) to give (**194**) as a colourless oil (2.4g, 28%); $[\alpha]_D$ +12.6 (*c* 2.7, $CHCl_3$); (Found: C, 68.9; H, 6.91. Calc. for $C_{30}H_{36}O_8$: C, 68.67; H, 6.92%); δ_H ($CDCl_3$; 270 MHz) 2.36 (1 H, br s, exch, D_2O, OH), 3.38 (3 H, s, OCH_3), 3.48–3.79 (6 H, m, 2-H, 3-H, 4-H, 5-H, 6-H, 6-H'), 3.78, 3.79 (6 H, 2 s, 2 x OCH_3), 4.43–4.93 (7 H, m, 1-H, 3 x ArCH_2O AB systems), 6.83–6.90 (4 H, m, 3-H and 5-H of PMB rings), 7.22–7.36 (9 H, m, aromatic CH); δ_C ($CDCl_3$; 67.8 MHz) 55.14, 55.17 (2 x OCH_3), 69.18 (6-C), 69.82, 70.66 (4-C, 5-C), 73.08, 73.16, 74.96 (3 x ArCH_2O), 79.53, 81.02 (2-C, 3-C), 98.12 (1-C), 113.70, 113.91 (2 x 3-C and 5-C of PMB rings), 127.84, 128.02, 128.38, 129.21, 129.59 (aromatic CH), 130.02, 130.91 (2 x 1-C of PMB rings), 138.04 (1-C of benzyl ring), 159.14, 159.26 (2 x 4-C of PMB rings); *m/z* (FAB)$^-$ 523 [(M-1)$^-$, 70%], 677 [(M+NBA)$^-$,80%].

A sample of (**194**) was converted to its syrupy 4-*O*-benzoyl ester (**195**); $[\alpha]_D$ −12.1 (*c* 2.2, $CHCl_3$); δ_H ($CDCl_3$; 270 MHz) 3.43 (3 H, s, OCH_3), 3.42–3.54 (2 H, m, 6-H, 6-H'), 3.62–3.67 (4 H, m, OCH_3, 2-H), 3.70 (3 H, s, OCH_3), 3.94 (1 H, ddd, *J* 3.5, 9.2, 9.8, 5-H), 4.03 (1 H, t, *J* 9.5, 3-H), 4.37, 4.41 (2 H, AB, J_{AB} 13.1, ArCH_2O), 4.53–4.85 (5 H, m, 1-H, 2 x ArCH_2O AB systems) 5.28 (1 H, t, *J* 9.7, 4-H), 6.59 (2 H, d, *J* 8.6, 3-H and 5-H of PMB ring), 6.71 (2 H, d, *J* 8.6, 3-H and 5-H of PMB ring), 7.04 (2 H, d, *J* 8.6, 2-H and 6-H of PMB ring), 7.15 (2 H, d, *J* 8.6, 2-H and 6-H of PMB ring), 7.25–7.56 (8 H, m, aromatic CH), 7.90–7.94 (2 H, m, 2-H and 6-H of benzoyl ring); *m/z* (FAB$^+$) 628 [(M+1)$^+$, 10%].

Further elution (eluent hexane : ethyl acetate 2:3) gave (**185**) as a pale yellow oil which solidified to a waxy solid (m.p. 50–51.5°C) on standing (4.1g, 48%); $[\alpha]_D$ +7.5 (*c* 1.1,

$CHCl_3$); (Found: C, 68.6; H, 7.05. Calc. for $C_{30}H_{36}O_8$: C, 68.67; H, 6.92%); δ_H ($CDCl_3$; 400 MHz) 1.85 (1 H, br s, exch. D_2O, OH), 3.35 (3 H, s, OCH_3), 3.45–3.50 (2 H, m, 2-H, 4-H), 3.59–3.76 (3 H, m, 5-H, 6-H, 6-H'), 3.78, 3.79 (6 H, 2 s, 2 x $ArOCH_3$), 3.97 (1 H, t, *J* 9.3, 3-H), 4.55–4.99 (7 H, m, 1-H, 3 x $ArCH_2O$ AB systems), 6.84–6.88 (4 H, m, 2 x 3-H and 5-H of PMB rings), 7.20–7.38 (9 H, m, aromatic CH); δ_C ($CDCl_3$; 67.8 MHz) 55.07, 55.19 (2 x OCH_3), 61.84 (6-C), 70.64 (5-C), 73.35, 74.57, 75.35 (3 x $ArCH_2O$), 77.47, 79.98, 81.67 (2-C, 3-C, 4-C), 98.12 (1-C), 113.77, 113.83 (2 x 3-H and 5-H of PMB rings), 127.84, 128.02, 128.39, 129.55, 129.59 (aromatic CH), 130.29, 130.94 (2 x 1-C of PMB rings), 138.12 (1-C of benzyl ring), 159.14, 159.29 (2 x 4-C of PMB rings); *m/z* (FAB^-) 677 [$(M+NBA)^-$, 60%].

A sample of (**185**) was converted to its 6-*O*-benzoyl ester (**196**), R_f 0.64 (hexane : ethyl acetate 2:3); m.p. 120.5°C (from isopropanol); $[\alpha]_D$ +86.2 (*c* 1.2, $CHCl_3$); (Found: C, 70.9; H, 6.30. Calc. for $C_{37}H_{40}O_9$: C, 70.68; H, 6.41%); δ_H ($CDCl_3$; 270 MHz) 3.38 (3 H, s, OCH_3), 3.54–3.62 (2 H, m, 2-H, 4-H), 3.72, 3.81 (6 H, 2 s, 2 x $ArOCH_3$), 3.92 (1 H, dq, *J* 2, 9.6, 5-H), 4.03 (1 H, t, *J* 9.2, 3-H), 4.46–4.97 (9 H, m, 3 x $ArCH_2O$ AB systems, 1-H, 6-H, 6-H'), 6.80 (2 H, d, *J* 8.6, 3-H and 5-H of PMB ring), 6.89 (2 H, d, *J* 8.6, 3-H and 5-H of PMB ring),7.17–8.14 (14 H, m, aromatic CH); *m/z* (FAB^+) 628 [M^+, 5%].

b) To a solution of (**190**) (5.7g, 10.9mmol) in freshly distilled, dry THF (200cm^3) was added $LiAlH_4$ (2g) in one portion. The mixture was gradually brought to refluxing temperature. After 1 h, aluminium chloride (6g) was added portionwise over 30 min (*care!*). The mixture was stirred under reflux for a further 2 h, then cooled to 0°C. Excess $LiAlH_4$ was destroyed by careful addition of ethyl acetate (20cm^3) and aluminium hydroxide was precipitated by addition of water (30cm^3). The system was extracted with ether (300cm^3) and the combined organic extracts were washed with water (200cm^3), dried ($MgSO_4$), filtered and concentrated. The syrup thus obtained was purified by flash chromatography (eluent hexane : ethyl acetate 7:3) to give exclusively (**185**) (4.2g, 73%).

6.3.6 Methyl 2-*O*-benzyl-3,4-di-*O*-(*p*-methoxybenzyl)-α-D-*gluco*-hexodialdo-pyranoside (1,5) (205)

Dry DMSO (0.33cm^3) was added dropwise to a 2M solution of oxalyl chloride in dry dichloromethane (1.25cm^3 , 2.5mmol) at –60°C under an atmosphere of nitrogen. This mixture was stirred for 5 min, when a solution of (**185**) (1.2g, 2.3mmol) in dry dichloromethane (5cm^3) was added dropwise over 5 min. The mixture was stirred at –60°C for 20 min when triethylamine (1.2cm^3, 9.2mmol) was added. After a further 10 min, the mixture was allowed to warm to room temperature. The solvents were evaporated and the residue was subjected to flash chromatography (eluent ethyl acetate : hexane 3:2) to give the crude product which showed OH stretching at ν_{max} 3480cm^{-1} in the IR spectrum. The product was dissolved in toluene (150cm^3) and heated under reflux for 3 h with continuous azeotropic removal of water (Dean-Stark trap). The solution was cooled and concentrated to give the pure title compound as a pale yellow oil (1.0g, 84%); R_f 0.62 with streaking (ethyl acetate); $[\alpha]_D$ +19.6 (*c* 4.0, $CHCl_3$); (Found: C, 68.7; H, 6.63. Calc. for $C_{30}H_{34}O_8$: C, 68.94; H, 6.56%); δ_H ($CDCl_3$;400 MHz) 3.36 (3 H, s, OCH_3), 3.47 (1 H, dd, *J* 3.4, 9.3, 2-H), 3.53 (1 H, dd, *J* 8.8, 10.3, 4-H), 3.79, 3.80 (6 H, 2 s, 2 x $ArOCH_3$), 4.05 (1 H, t, *J* 9.3, 3-H), 4.13 (1 H, d, *J* 10.3, 5-H), 4.53–4.93 (7 H, m, 3 x $ArCH_2O$ AB systems, 1-H), 6.85–6.89 (4 H, m, 3-H and 5-H of PMB rings), 7.15–7.36 (9 H, m, aromatic CH), 9.62 (1 H, s, 6-H); δ_C ($CDCl_3$;100MHz) 55.26, 55.74 (2 x OCH_3), 73.63 ($ArCH_2O$), 74.25 (5-C), 74.73, 75.64 (2 x $ArCH_2O$), 77.45, 79.30, 81.51 (2-C, 3-C, 4-C), 98.41 (1-C), 113.90 (3-C and 5-C of PMB rings), 128.11, 128.18, 128.47, 128.57, 129.04, 129.57, 129.68, 129.82 (aromatic CH), 129.88, 130.63 (2 x 1-C of PMB rings), 137.87 (1-C of benzyl ring), 159.31, 159.49 (2 x 4-C of PMB rings), 197.58 (6-C); *m/z* : (FAB$^-$) 677 [(M+NBA)$^-$, 60%]; ν_{max} 1745cm^{-1}.

6.3.7 (1*R*, 2*S*, 3*S*, 4*R*, 5*S*)-3-Benzyloxy-4-hydroxy-5-hydroxymethyl-1,2-di-(*p*-methoxybenzyloxy)cyclopentane (206)

The method employed is a modification of that described by Chénedé *et al.*[260] To a 0.1M solution of samarium (II) iodide in dry THF (100cm^3, 10mmol) under an atmosphere of nitrogen were added dry HMPA (5cm^3) and freshly distilled, dry *t*-

butanol (0.4cm^3, 4.3mmol). A solution of (**205**) (926mg, 1.8mmol) in freshly distilled, dry THF (20cm^3) was then added dropwise over 15 min and the blue-black mixture was stirred under a stream of nitrogen for a further 1 h. 1M Aqueous HCl (100cm^3) was added and the mixture was extracted with ether (3 x 150cm^3). The combined organic extracts were washed with 5%^{w}/$_v$ aqueous $Na_2S_2O_3$ solution (100cm^3) and water (100cm^3), dried ($MgSO_4$), filtered and concentrated. The residual orange oil was subjected to flash chromatography (eluent hexane : ethyl acetate 3:2) to give (**185**) (175mg, 19%);

Further elution with ethyl acetate gave (**206**) as a pale yellow oil (321mg, 37%); R_f 0.49 (ethyl acetate); $[\alpha]_D$ +19.5 (*c* 4.1, $CHCl_3$); (Found: C, 70.5; H, 6.57. Calc. for $C_{29}H_{34}O_7$: C, 70.41; H, 6.93%); δ_H ($CDCl_3$;400MHz) 2.24 (1 H, m, 5-H), 2.50–3.40 (2 h, br s, 2 x OH), 3.72–3.77 (7 H, m, 2 x $ArOCH_3$, 3-H), 3.78–3.83 (2 H, m, CH_2OH), 3.90 (1 H, t, *J* 5.4, 2-H), 3.97 (1 H, t, *J* 5.9, 1-H), 4.24 (1 H, dd, *J* 3.9, 6.8, 4-H), 4.47–4.65 (6 H, m, 3 x $ArCH_2O$ AB systems), 6.83–6.86 (4 H, m, 3-H and 5-H of PMB rings), 7.20–7.33 (9 H, m, aromatic CH); δ_C ($CDCl_3$;100MHz) 46.68 (5-C), 55.18 (OCH_3), 60.93 (CH_2OH), 71.72, 71.81 (3 x $ArCH_2O$), 75.20, 81.66, 87.42, 87.90 (1-C, 2-C, 3-C, 4-C), 113.70 (3-C and 5-C of PMB rings), 127.68, 127.75, 128.35, 129.41, 129.54 (aromatic CH), 130.05, 130.31 (2 x 1-C of PMB rings), 138.06 (1-C of benzyl ring), 159.13, 159.17 (2 x 4-C of PMB rings); *m/z*: (FAB$^-$) 493 [(M+1)$^-$, 50%].

6.3.8 (1*R*, 2*S*, 3*S*, 4*R*, 5*S*)-3-Benzyloxy-5-benzyloxymethyl-4-hydroxy-1,2-di-(*p*-methoxybenzyloxy)cyclopentane (208) and (1*R*, 2*S*, 3*S*, 4*R*, 5*S*)-3,4-Dibenzyloxy-5-hydroxymethyl-1,2-di-(*p*-methoxybenzyloxy)cyclopentane (207)

A solution of (**206**) (200mg, 0.4mmol) in dry DMF (3cm^3) at 0°C was sequentially treated with sodium hydride (17mg of a 60% ^{w}/$_w$ dispersion in mineral oil, 0.4mmol) and benzyl bromide (0.05cm^3, 0.45mmol). The mixture was stirred at 0°C for 1 h, when TLC (ethyl acetate : hexane 3:2) indicated two products (R_f 0.58 and 0.49) and unreacted starting material (R_f 0.15). Methanol (1cm^3) was added and the solution was stirred for a further 5 min. The solvents were evaporated and the residue was extracted with chloroform (100cm^3). The organic solution was washed with water (100cm^3) and saturated aqueous NaCl solution (100cm^3), dried ($MgSO_4$), filtered and concentrated.

The syrupy residue was subjected to flash chromatography (eluent chloroform : acetone 30:1) to give (**208**) as a pale yellow oil (67mg, 28%); $[\alpha]_D$ +30.4 (*c* 2.0, $CHCl_3$); (Found: C, 73.8; H, 7.00. Calc. for $C_{36}H_{40}O_7$: C, 73.94; H, 6.90%); δ_H ($CDCl_3$;400MHz) 2.31 (1 H, m, *J* 4.9, 9.3, 5-H), 3.32 (1 H, d, *J* 3.4, OH), 3.72–3.78 (2 H, m, C*H*$_2$OCH$_2$Ph), 3.77–3.81 (7 H, m, 2 x OCH_3, 3-H), 3.90 (1 H, t, *J* 5.9, 2-H), 4.06 (1 H, dd, *J* 5.9, 9.3, 1-H), 4.25 (1 H, br m, 4-H), 4.40–4.70 (8 H, m, 4 x ArC*H*$_2$O AB systems), 6.81–6.87 (4 H, m, 2-H and 5-H of PMB rings), 7.15–7.37 (14 H, m, aromatic CH); δ_C ($CDCl_3$;100MHz) 45.41 (5-C), 55.27 (OCH_3), 67.98 (*C*H$_2$OCH$_2$Ph), 71.69, 71.78, 72.00, 73.52 (4 x ArCH$_2$O), 75.07, 81.32, 88.04 (4 x CH), 113.73 (2-C and 5-C of PMB rings), 127.63, 127.76, 127.82, 127.89, 128.36, 128.44, 128.49, 129.55, 129.63 (aromatic CH), 130.38, 130.60 (2 x 1-C of PMB rings), 137.51, 138.24 (2 x 1-C of benzyl rings), 159.16, 159.20 (2 x 4-C of PMB rings); *m/z*: (FAB^+) 463 [$(M\text{-}PMB)^+$, 65%].

Further elution gave (**207**) as a pale yellow oil (98mg, 41%); $[\alpha]_D$ +27.9 (*c* 1.5, $CHCl_3$); (Found: C, 73.7; H, 6.92. Calc. for $C_{36}H_{40}O_7$: C, 73.94; H, 6.90%); δ_H ($CDCl_3$;400MHz) 2.33 (1 H, m, *J* 5.9, 5-H), 2.57 (1 H, br m, exch. D_2O, OH), 3.77, 3.78 (6 H, 2 s, 2 x OCH_3), 3.82–3.87 (2 H, m, C*H*$_2$OH), 3.88–3.91 (1 H, br m, 3-H), 3.95 (1 H, t, *J* 5.9, 2-H), 4.00–4.03 (1 H, br m, 1-H), 4.12 (1 H, dd, *J* 5.9, 8.8, 4-H), 4.46–4.66 (8 H, m, 4 x ArC*H*$_2$O AB systems), 6.84–6.87 (4 H, m, 3-H and 5-H of PMB rings), 7.23–7.35 (14 H, m, aromatic CH);); δ_C ($CDCl_3$;100MHz) 46.78 (5-C), 55.25 (OCH_3), 60.95 (*C*H$_2$OH), 71.78, 71.85, 72.19 (4 x ArCH$_2$O), 82.24, 82.42, 85.77, 88.37 (1-C, 2-C, 3-C, 4-C), 113.77 (3-C and 5-C of PMB rings), 127.63, 127.74, 127.96, 128.42, 128.49, 128.57, 129.44, 129.53, 129.63 (aromatic CH), 130.27, 130.47 (2 x 1-C of PMB rings), 137.64, 138.04 (2 x 1-C of benzyl rings), 159.20, 159.23 (2 x 4-C of PMB rings); *m/z*: (FAB^+) 463 [$(M\text{-}PMB)^+$, 15%].

Further elution with ethyl acetate gave starting material (50mg, 25%).

6.3.9 (1*R*, 2*S*, 3*S*, 4*R*, 5*R*)-3-Benzyloxy-5-benzyloxymethyl-1,2,4-trihydroxycyclopentane (202)

A solution of (**208**) (90mg, 0.15mmol) in ethanol–1M aqueous HCl (2:1, 30cm^3) was heated under reflux for 2.5 h, when TLC (ethyl acetate) indicated consumption of starting material (R_f 0.79) to give a product (R_f 0.31) The solution was cooled, the solvents were evaporated and the residue was triturated with ether (3 x 50cm^3). The combined organic solutions were washed with saturated aqueous $NaHCO_3$ solution (50cm^3) and saturated aqueous NaCl solution (50cm^3), dried ($MgSO_4$), filtered and concentrated. The residue was purified by flash chromatography (eluent hexane : ethyl acetate 1:1) to give the title compound as a waxy solid (38mg, 72%); $[\alpha]_D$ +30.0 (*c* 1.7, $CHCl_3$); δ_H ($CDCl_3$;400MHz) 2.16–2.23 (1 H, br m, 5-H), 3.14 (1 H, br s, OH), 3.43 (1 H, br s, OH), 3.61 (1 H, dd, *J* 3.4, 6.1, 3-H), 3.67–3.74 (3 H, m, C*H*$_2$OCH$_2$Ph, OH), 3.82 (1 H, br t, *J* 6.5, 2-H), 3.95 (1 H, br dd, *J* 7.6, 1-H), 4.14–4.18 (1 H, br m, 4-H), 4.46, 4.48 (2 H, AB, J_{AB} 11.9, PhC*H*$_2$O), 4.60, 4.67 (2 H, AB, J_{AB} 11.6, PhC*H*$_2$O), 7.23–7.35 (10 H, m, aromatic CH); δ_C ($CDCl_3$;100 MHz) 45.90 (5-C), 68.05 (*C*H$_2$OCH$_2$Ph), 71.96, 73.52 (2 x PhCH$_2$O), 74.47, 75.97, 81.02 (1-C, 2-C, 4-C), 89.19 (3-C), 127.76, 127.91, 128.00, 128.44, 128.58 (aromatic CH), 137.45, 138.02 (2 x 1-C of benzyl rings); *m/z*:(FAB$^+$) 345 [(M+1)$^+$, 15%], (FAB$^-$) 343 [(M-1)$^-$, 38%], 497 [(M+NBA)$^-$, 98%].

6.3.10 (1*R*, 2*R*, 3*S*, 4*R*, 5*S*)-3-Hydroxy-5-hydroxymethyl-1,2,4-trisphosphocyclopentane (200)

A mixture of bis(benzyloxy)(diisopropylamino)phosphine (211mg, 6.1mmol), 1*H*-tetrazole (64mg, 9.2mmol) and dry dichloromethane (2cm^3) was vigorously stirred at room temperature for 15 min, whereupon a solution of (**202**) (35mg, 0.1mmol) in dry dichloromethane (1cm^3) was added and stirring was continued for 30 min. ^{31}P NMR spectroscopy indicated phosphite triester signals at δ_P 140.3 (s) and 140.2, 139.9 (AB, J_{AB} 7.0). The mixture was cooled to –78°C, MCPBA (312mg) was added and the system was allowed to warm to room temperature. After a further 10 min, ethyl acetate (50cm^3) was added and the organic solution was washed with 50cm^3 each of 10%^{w}/$_{v}$ aqueous $Na_2S_2O_3$ solution, 1M aqueous HCl, saturated aqueous $NaHCO_3$ solution and

saturated aqueous NaCl solution. The organic fraction was dried ($MgSO_4$), filtered and concentrated. Flash chromatography with chloroform : acetone 20:1 and recolumning with ethyl acetate : hexane 7:3 then 1:1 failed to remove all impurities. ^{31}P NMR spectroscopy showed three peaks at δ_P –2.47, –2.16 and –2.08 and this crude product was used for deprotection.

Ammonia (*ca.* 100cm^3) was condensed into a three-necked flask at –78°C. An excess of sodium was added to dry the liquid ammonia, about 20cm^3 of which was then distilled into a second three-necked flask and kept at –78°C. Sodium was added until the solution remained blue-black for 10 min. A solution of the crude product above in dry dioxane (1cm^3) was added. The mixture was stirred for 2 min then the reaction was quenched with methanol (1cm^3) and water (1cm^3). The solvents were evaporated. The residue was dissolved in de-ionised water (300cm^3) and purified by ion-exchange chromatography on Q Sepharose fast flow, eluting with a gradient of triethylammonium bicarbonate buffer (0–1M), pH 7.3. The triethylammonium salt of the title compound eluted between 610–640mM buffer; $[\alpha]_D$ –11.0 (*c* 0.4 calc. for free acid, TEAB, pH 8.6); δ_H (D_2O, pH *ca.* 4, 400 MHz) 2.29 (1 H, br quintet, *J* 7.3, 5-H), 3.65 (1 H, ABX, $^2J_{AB}$ 12, ^3J 6, C*H*HOH), 3.71 (1 H, ABX, $^2J_{AB}$ 12, ^3J 7.3, CH*H*OH), 3.94 (1 H, t, *J* 4.6, 3-H), 4.16–4.31 (3 H, m, 1-H, 2-H, 4-H); δ_P (D_2O, pH *ca.* 4, 400 MHz) (^{1}H-coupled) 0.36 (d, J_{HP} 9.3), 0.49 (d, J_{HP} 8.8), 0.66 (d, J_{HP} 9.2); *m/z*: (FAB$^-$) 403 [(M-1)$^-$, 100%]; (Found: M$^-$, 402.962. Calc. for $C_6H_{12}O_{14}P_3$ [M-H]$^-$: 402.960).

6.4 Attempted Preparation of a Versatile Chiral Intermediate for Synthesis of Analogues of Ins(1,4,5)P_3

6.4.1 Methyl 2-*O*-(*p*-methoxybenzyl)-α-D-glucopyranoside (214)

a) The method employed is a modification of that described by Haque *et al.*[262] A mixture of methyl α-D-glucopyranoside (20.0g, 103mmol) and dibutyltin oxide (25.6g, 103mmol) was heated under reflux in methanol (500cm^3) until solution was effected (3.5 h). The solution was cooled and concentrated. To the foamy residue was added tetrabutylammonium iodide (38.0g, 103mmol), acetonitrile (500cm^3) and *p*-methoxybenzyl chloride (15.5cm^3, 123mmol). This mixture was heated under reflux *via* a Soxhlet thimble containing 4Å molecular sieves for 16 h, whereupon more *p*-methoxybenzyl chloride (3.0cm^3, 23.8mmol) was added, and heating was continued for a further 5 h. The solution was cooled and concentrated. Without further purification the residue was fractionated by flash chromatography (eluent ethyl acetate : hexane 4:1 then ethyl acetate) to give a less polar fraction (R_f>0.55 in ethyl acetate : methanol 4:1) and a more polar fraction (R_f 0.35–0.48 in the same solvent system). The latter was collected and the title compound was isolated by fractional crystallisation from ethanol (yield 11.8g, 36%); R_f 0.40 (ethyl acetate : methanol 4:1); m.p. 136–138°C; $[\alpha]_D$ +61.3 (*c* 0.8, $CHCl_3$); (Found: C, 57.1; H, 7.13. Calc. for $C_{15}H_{22}O_7$: C, 57.32; H, 7.05%); δ_H ($CDCl_3$; 270 MHz) 2.20 (1 H, br s, exch. D_2O, OH), 2.74 (1 H, br s, exch. D_2O, OH), 3.32 (3 H, s, OCH_3), 3.45–3.55 (2 H, m, 6-H, 6-H'), 3.67 (1 H, br s, exch. D_2O, OH), 3.79 (3 H, s, $ArOCH_3$), 3.81–3.95 (4 H, br m, 2-H, 3-H, 4-H, 5-H), 4.53–4.67 (3 H, m, $ArCH_2O$ AB system overlapping 1-H), 6.87 (2 H, d, *J* 8.7, 3-H and 5-H of PMB ring), 7.28 (2 H, d, *J* 8.7, 2-H and 6-H of PMB ring); δ_C ($CDCl_3$; 67.8 MHz) 55.29 (OCH_3), 61.74 (6-C), 70.14, 70.86 (CH), 72.75 ($ArCH_2O$), 79.01 (2-C), 97.97 (1-C), 113.98 (3-C and 5-C of PMB ring), 129.79 (2-C and 6-C of PMB ring), 130.07 (1-C of PMB ring), 159.53 (4-C of PMB ring); *m/z* (FAB^-) 313 [$(M-1)^-$, 90%].

b) A solution of (**163**) (16.2g, 39.8mmol) in 80%$^{v/v}$ acetic acid in water (100cm^3) was heated under reflux for 30 min. The solution was rapidly cooled (ice bath) and the solvents were evaporated and co-evaporated with toluene (3 x 100cm^3). The residue

thus obtained was extracted with water (3 x 200cm^3) and the combined aqueous extracts were washed with light petroleum (100cm^3) to remove benzaldehyde, and concentrated to give the title compound as a white solid (11.6g, 93%).

6.4.2 Methyl 4,6-*O*-isopropylidene-2-*O*-(*p*-methoxybenzyl)-α-D-glucopyranoside (215)

A solution of (**214**) (1.2g, 3.7mmol) in acetone was treated with PTSA (52mg) and 2-methoxypropene (3.5cm^3, 37mmol) and stirred for 5 min. The solvents were evaporated and the dark brown residue was extracted with ether (200cm^3). The organic extract was washed with saturated aqueous $NaHCO_3$ solution (70cm^3) and water (70cm^3), dried ($MgSO_4$), filtered and concentrated to give an orange oil which was purified by flash chromatography (eluent hexane : ethyl acetate 7:3 then 2:3) to give the title compound as a pale yellow oil (920mg, 71%); R_f 0.52 (ethyl acetate); $[\alpha]_D$ +25.0 (*c* 2.4, $CHCl_3$); (Found: C, 60.8; H, 7.63. Calc. for $C_{18}H_{26}O_7$: C, 61.00; H, 7.39%); δ_H ($CDCl_3$; 400MHz) 1.44, 1.50 (6 H, 2 s, 2 x isopropylidene CH_3), 2.58 (1 H, br s, exch. D_2O, OH), 3.34 (3 H, s, OCH_3), 3.39 (1 H, dd, *J* 3.4, 9.3, 2-H), 3.53 (1 H, t, *J* 9.3, 4-H), 3.62 (1 H, td, $^3J_{4\text{-H, 5-H}} = {}^3J_{6\text{-Hax, 5-H}} = 9.3$, $^3J_{6\text{-Heq, 5-H}} = 4.9$, 5-H), 3.71 (1 H, t, $^3J_{6\text{-Hax,5-H}} = {}^2J_{6\text{-Hax, 6-Heq}} = 9.8$, 6-$H_{ax}$), 3.81–3.86 (4 H, m, $ArOCH_3$, 6-H_{eq}), 3.96 (1 H, br t, *J* 9.3, 3-H), 4.53 (1 H, d, *J* 3.9, 1-H), 4.61, 4.68 (2 H, AB, J_{AB} 12, $ArCH_2O$), 6.89 (2 H, d, *J* 8.3, 3-H and 5-H of PMB ring), 7.30 (2 H, d, *J* 8.7, 4-H and 6-H of PMB ring); δ_C ($CDCl_3$; 67.8 MHz) 19.07 (axial CH_3 of isopropylidene), 29.07 (equatorial CH_3 of isopropylidene), 55.22 (OCH_3), 62.37 (6-C), 62.87, 70.29 (CH), 72.83 ($ArCH_2O$), 73.72, 79.35 (CH), 98.52 (1-C), 99.67 (C_q of isopropylidene), 113.94 (3-C and 5-C of PMB ring), 129.70 (2-C and 6-C of PMB ring), 129.93 (1-C of PMB ring), 159.52 (4-C of PMB ring); *m/z* (FAB$^-$) 353 [(M-H)$^-$, 40%].

6.4.3 Methyl 3,4-*O*-isopropylidene-2-*O*-(*p*-methoxybenzyl)-α-D-glucopyranoside (210)

A solution of (**214**) (17.1g, 54.5mmol), DMAP (280mg, 2.3mmol) and *t*-butyldiphenylsilyl chloride (14.9cm^3, 57.2mmol) in dry pyridine (100cm^3) was left at room temperature for 16 h, when TLC (ethyl acetate) indicated consumption of starting

material (R_f 0.11) to give a single product (R_f 0.53). The solvents were evaporated and co-evaporated with toluene (3 x 250cm^3) to remove remaining traces of pyridine. To the residue was added acetone (200cm^3), PTSA (100mg) and 2-methoxypropene (26cm^3, 273mmol). After 4 h, TLC indicated the presence of a single product (R_f 0.68 in ethyl acetate). The solvents were evaporated and the residue was heated under reflux in a 1.1M solution of tetrabutylammonium fluoride in THF (240cm^3, 218mmol) for 16 h, when TLC indicated a single product (R_f 0.44 in ethyl acetate). The solution was cooled and poured into a separating funnel containing water (350cm^3). The resulting emulsion was extracted with ether (3 x 250cm^3) and the combined organic extracts were dried ($MgSO_4$), filtered and concentrated. The resultant orange oil was subjected to flash chromatography (eluent hexane : ethyl acetate 1:1 then 1:4) to give the title compound as a pale yellow oil (18.2g, 95%); $[\alpha]_D$ –5.9 (*c* 0.9, $CHCl_3$); (Found: C, 60.9; H, 7.61. Calc. for $C_{18}H_{26}O_7$: C, 61.00; H, 7.39%); δ_H ($CDCl_3$; 400MHz) 1.45, 1.46 (6 H, 2 s, 2 x isopropylidene CH_3), 2.02 (1 H, br s, exch. D_2O, OH), 3.25 (1 H, t, *J* 9.3, 4-H), 3.36 (3 H, s, OCH_3), 3.63 (1 H, dd, *J* 3.4, 9.4, 2-H), 3.73 (1 H, br m, sharpens on D_2O exch., 6-H), 3.81 (3 H, s, $ArOCH_3$), 3.82–3.89 (2 H, m, 5-H, 6-H'), 3.96 (1 H, t, *J* 9.3, 3-H), 4.58 (1 H, AB, J_{AB} 12.2, ArC*H*HO), 4.65 (1 H, d, *J* 3.4, 1-H), 4.81 (1 H, AB, J_{AB} 12.2, ArCH*H*O), 6.89 (2 H, d, *J* 8.8, 3-H and 5-H of PMB ring), 7.31 (2 H, d, *J* 8.8, 2-H and 6-H of PMB ring); δ_C ($CDCl_3$; 100MHz) 26.52, 26.96 (2 x isopropylidene CH_3), 55.52, 55.29 (2 x OCH_3), 62.55 (6-C), 71.60 (CH), 71.78 (ArCH_2O), 74.45, 77.40, 77.61 (CH), 99.07 (1-C), 111.01 (C_q of isopropylidene), 113.81 (3-C and 5-C of PMB ring), 129.88 (2-C and 6-C of PMB ring), 130.03 (1-C of PMB ring), 159.40 (4-C of PMB ring); *m/z* (FAB$^+$) 355 [(M+1)$^+$, 2%].

6.4.4 (*E*,*Z*)-Methyl 6-*O*-acetyl-3,4-*O*-isopropylidene-2-*O*-(*p*-methoxybenzyl)-α-D-*xylo*-hex-5-enopyranoside (216ab) and methyl 6-*C*-acetoxy-6-*O*-acetyl-3,4-*O*-isopropylidene-2-*O*-(*p*-methoxybenzyl)-α-D-glucopyranoside (217)

a) Dry DMSO (1.6cm^3, 22.6mmol) was added dropwise to a 2M solution of oxalyl chloride in dry dichloromethane (6.2cm^3, 12.4 mmol) at –60°C ($CO_{2(s)}$ /$CHCl_3$ bath) under an atmosphere of nitrogen. After 5 min a solution of (**210**) (4.0g, 11.3mmol) in dry dichloromethane (5cm^3) was added over about 5 min. The mixture was stirred at –60°C for 15 min, then *N*,*N*-diisopropylethylamine (7.9cm^3, 45.2mmol) was added over

5 min. The system was allowed to warm to room temperature and the solvents were evaporated. To the residue was added anhydrous potassium carbonate (9.4g, 67.8mmol, dried *in vacuo* over P_2O_5 at 75°C for 6 h), acetic anhydride (10.7 cm^3, 113mmol) and dry acetonitrile (30cm^3), and this mixture was heated under reflux under an atmosphere of nitrogen for 6 h, then cooled. The mixture was extracted with ether (2 x 200cm^3) and the combined organic extracts were washed with saturated aqueous KCl solution (200cm^3) and water (200cm^3), dried ($MgSO_4$), filtered and concentrated. The brown oil thus obtained was subjected to flash chromatography (eluent hexane : ethyl acetate 7:3) to give (**216ab**) as a pale yellow solid (1.2g, 26%); R_f 0.75 (ethyl acetate); $[\alpha]_D$ –45.1 (*c* 1.6, $CHCl_3$); (Found: C, 60.8; H, 6.69. Calc. for $C_{20}H_{26}O_8$: C, 60.89; H, 6.65%); δ_H (major isomer) ($CDCl_3$; 270 MHz) 1.47, 1.49 (6 H, 2 s, 2 x isopropylidene CH_3), 2.16 (3 H, s, CH_3CO_2), 3.47 (3 H, s, OCH_3), 3.74–3.80 (2 H, m, 2-H, 4-H), 3.81 (3 H, s $ArOCH_3$), 3.96 (1 H, t, *J* 9, 3-H), 4.57–4.87 (3 H, m, $ArCH_2O$ AB system overlapping with 1-H), 6.89 (2 H, d, *J* 8.8, 3-H and 5-H of PMB ring), 6.95 (1 H, d, *J* 1.7, 6-H), 7.31 (2 H, d, *J* 8.8, 4-H and 6-H of PMB ring); δ_C (major isomer) ($CDCl_3$; 68MHz) 20.54 (CH_3CO_2), 26.41, 26.98 (2 x CH_3 of isopropylidene), 55.29, 56.38 (2 x OCH_3), 71.98 ($ArCH_2O$), 73.39, 76.86, 77.94 (2-C, 3-C, 4-C), 101.32 (1-C), 112.18 (C_q of isopropylidene), 113.86 (3-C and 5-C of PMB ring), 118.93 (6-C), 129.90 (1-C of PMB ring), 129.96 (2-C and 6-C of PMB ring), 133.70 (5-C), 159.49 (4-C of PMB ring), 167.21 (CH_3CO_2); *m/z* (FAB^-) 393 [$(M-1)^-$, 40%].

Further elution gave (**217**) as a colourless oil (2.2g, 43%); R_f 0.70 (ethyl acetate); $[\alpha]_D$ –21.1 (*c* 1.8, $CHCl_3$); (Found: C, 58.2; H, 6.83. Calc. for $C_{22}H_{30}O_{10}$: C, 58.13; H, 6.66%); δ_H ($CDCl_3$; 400MHz) 1.42, 1.43 (6 H, 2 s, 2 x isopropylidene CH_3), 2.08, 2.11 (6 H, 2 s, 2 x CH_3CO_2), 3.27 (1 H, t, *J* 9.8, 4-H), 3.35 (3 H, s, OCH_3), 3.64 (1 H, dd, *J* 3.4, 10.5, 2-H), 3.80 (3 H, s, $ArOCH_3$), 3.90–3.97 (2 H, m, 3-H, 5-H), 4.56 (1 H, AB, J_{AB} 12.2, ArCHHO), 4.65 (1 H, d, *J* 3.4, 1-H), 4.80 (1 H, AB, J_{AB} 12.2, ArCHHO), 6.88 (2 H, d, *J* 8.8, 3-H and 5-H of PMB ring), 6.95 (1 H, d, *J* 4.9, 6-H), 7.30 (2 H, d, *J* 8.8, 2-H and 6-H of PMB ring); δ_C ($CDCl_3$; 100 MHz) 20.67, 20.70 (2 x CH_3CO_2), 26.74, 26.85 (2 x isopropylidene CH_3), 55.27, 55.49 (2 x OCH_3), 70.28 (CH), 71.91 ($ArCH_2O$), 74.45, 76.77, 77.70, 87.93 (4 x CH), 98.96 (1-C), 111.12 (C_q of isopropylidene), 113.83 (3-C and 5-C of PMB ring), 129.02 (1-C of PMB ring), 129.88 (2-C and 6-C of PMB

ring), 159.44 (4-C of PMB ring), 168.47, 168.62 (2 x CH_3CO_2); *m/z*: (FAB^+) 455 [$(M+1)^+$, 2%].

b) Compound (**210**) (4.5g, 12.7mmol) was oxidised as above. The residue present after evaporation of the solvents was subjected to flash chromatography (eluent hexane : ethyl acetate 1:1) and the partially purified aldehyde was dissolved in toluene (250cm^3). The solution was heated under reflux for 1.5 h with continuous azeotropic removal of water (Dean-Stark trap), then cooled and concentrated. The residue was treated as in *a)* to give (**216ab**) only (3.5g, 70%).

6.4.5 Attempted Ferrier rearrangement of (216ab)

To a solution of (**216ab**) (395mg, 1.0mmol) in acetone : water 4:1 (25cm^3) was added mercury (II) acetate (351mg, 1.1mmol). After 30 min TLC (ethyl acetate) indicated consumption of starting material (R_f 0.75) to give a baseline product. Sodium chloride (3.4g, 57mmol) was added and the suspension was stirred for 22 h. TLC indicated a single product (R_f 0.70 in ethyl acetate). The mixture was partially evaporated and diluted with chloroform (50cm^3). The organic extract was washed with water (30cm^3), dried ($MgSO_4$), filtered and concentrated. The residue was purified by flash chromatography (eluent hexane : ethyl acetate 7:3) to give 6-*O*-acetyl-3,4-*O*-isopropylidene-2-*O*-(*p*-methoxybenzyl)-D-*xylo*-5-hexosulose (**219**) as a pale yellow oil (124mg, 33%); $[\alpha]_D$ +23 (*c* 0.3, $CHCl_3$); δ_H ($CDCl_3$; 400MHz) 1.43, 1.45 (6 H, 2 s, 2 x isopropylidene CH_3), 2.17 (3 H, s, CH_3CO_2), 3.80 (3 H, s, $ArOCH_3$), 3.94 (1 H, dd, *J* 1.3, 2.9, 2-H), 4.46 (1-H, ABX, *J* 2.9, J_{AB} 7.6, 3-H), 4.54 (1 H, AB, J_{AB} 7.6, 4-H), 4.61, 4.70 (2 H, AB, J_{AB} 11.6, $ArCH_2O$), 4.86, 5.05 (2 H, AB, J_{AB} 18.0, 6-H, 6-H'), 6.88 (2 H, d, *J* 8.5, 3-H and 5-H of PMB ring), 7.28 (2 H, d, *J* 8.5, 2-H and 6-H of PMB ring), 9.64 (1 H, d, *J* 1.2, 1-H); δ_C ($CDCl_3$; 100MHz) 21.05 (CH_3CO_2), 26.26, 26.30 (2 x isopropylidene CH_3), 55.27 ($ArOCH_3$), 66.24 (6-C), 73.46 ($ArCH_2O$), 77.49, 79.02, 81.35 (3 x CH), 112.00 (C_q of isopropylidene), 114.04 (3-C and 5-C of PMB ring), 128.67 (1-C of PMB ring), 129.99 (2-C and 6-C of PMB ring), 159.75 (4-C of PMB ring), 171.18 (CH_3CO_2), 201.39 (1-C), 202.56 (5-C); *m/z* (FAB^-) 533 [$(M+NBA)^-$, 30%].

6.4.6 (*E,Z*)-Methyl 6-*O*-acetyl-2-*O*-benzyl-3,4-di-*O*-(*p*-methoxybenzyl)-α-D-*xylo*-hex-5-enopyranoside (224ab)

Compound (**185**) (12.6g, 24.0mmol) was oxidised to (**205**) as described in section 6.3.6. The crude aldehyde thus obtained was dissolved in dry acetonitrile (100cm^3) under an atmosphere of nitrogen. Potassium carbonate (20g) and acetic anhydride (24cm^3, 240mmol) were added and this suspension was heated under reflux for 12 h, then cooled. The system was extracted with ether (3 x 200cm^3) and the combined organic extracts were washed with saturated aqueous KCl solution (200cm^3), dried ($MgSO_4$), filtered and concentrated. The brown residue was purified by flash chromatography (eluent hexane : ethyl acetate 7:3) to give the title compound as a pale yellow syrup (11.7g, 86%); R_f 0.58 (hexane : ethyl acetate 2:3); $[\alpha]_D$ –39.4 (*c* 1.8, $CHCl_3$); (Found: C, 67.8; H, 6.36. Calc. for $C_{32}H_{36}O_9$: C, 68.06; H, 6.43%); δ_H (major isomer) ($CDCl_3$; 400MHz) 2.15 (3 H, s, $COCH_3$), 3.46 (3 H, s, OCH_3), 3.55 (1 H, dd, *J* 3.5, 9, 2-H), 3.79, 3.80 (6 H, 2 s, 2 x $ArOCH_3$), 3.89–3.94 (2 H, m, 3-H, 4-H), 4.63–4.86 (7 H, m, 3 x $ArCH_2O$ AB systems, 1-H), 6.85–6.89 (4 H, m, 2 x 3-H and 5-H of PMB rings), 7.16 (1 H, d, *J* 1, 6-H), 7.25–7.35 (9 H, m, aromatic CH); δ_C (major isomer) ($CDCl_3$; 68 MHz) 20.58 (CH_3CO_2), 55.16, 56.10 (OCH_3), 73.68, 74.13, 75.35 (3 x $ArCH_2O$), 79.01, 80.96 (CH), 99.75 (1-C), 113.73, 113.83 (2 x 3-C and 5-C of PMB rings), 122.95 (6-C), 123.03, 127.94, 128.04, 128.44, 129.61, 129.74 (aromatic CH), 130.71 (1-C of PMB ring[s]), 135.04 (5-C), 137.98 (1-C of benzyl ring), 159.18, 159.32 (2 x 4-C of PMB rings), 167.28 (CH_3CO_2); *m/z* (FAB$^+$) 564 [M$^+$, 5%].

6.4.7 D-1-*O*-Acetyl-3-*O*-benzyl-4,5-di-*O*-(*p*-methoxybenzyl)-*myo*-inos-6-ose (225)

A solution of (**224ab**) (11.2g, 19.9mmol) and mercury (II) trifluoroacetate (8.9g, 20.9mmol) 3:2 (300cm^3) was stirred at room temperature for 30 min, when sodium chloride (35g) was added and stirring was continued for 18 h. The system was partially evaporated and the gummy residue was extracted with chloroform (3 x 150cm^3). The combined organic extracts were washed with water (100cm^3), dried ($MgSO_4$), filtered and concentrated. The residue was subjected to flash chromatography (eluent hexane : ethyl acetate 7:3) and the crude product was crystallised from ethanol to give the title compound (5.3g, 36%); m.p. 150.5–153°C; $[\alpha]_D$ –44 (*c* 1.1, $CHCl_3$); (Found: C, 67.8;

H, 6.16. Calc. for $C_{31}H_{34}O_9$: C, 67.61; H, 6.23%); δ_H ($CDCl_3$; 400MHz) 2.23 (3 H, s, CH_3CO_2), 2.63 (1 H, s, exch. D_2O, OH), 3.79–3.85 (7 H, m, 2 x OCH_3, 3-H), 4.04 (1 H, t, *J* 9, 4-H), 4.12 (1 H, d, *J* 9.4, 5-H), 4.32 (1 H, br t, *J* 2.4, 2-H), 4.44–4.49 (1 H, AB, J_{AB} 11.0, ArC*H*HO), 4.69–4.87 (5 H, m, 3 x ArCH_2O AB systems), 5.15 (1 H, d, *J* 2.4, 1-H), 6.84–6.86 (4 H, m, 2 x 3-H and 5-H of PMB rings), 7.22–7.34 (9 H, m, aromatic CH); δ_C ($CDCl_3$; 100MHz) 20.54 (CH_3CO_2), 55.27 (OCH_3), 69.28 (CH), 73.23, 73.37 (2 x ArCH_2O), 74.93 (CH), 75.82 (ArCH_2O), 78.91, 81.49, 83.21 (3-C, 4-C, 5-C), 113.75, 113.83 (2 x 3-H and 5-H of PMB rings), 127.98, 128.18, 128.62 (aromatic CH), 129.45 (1-C of PMB ring), 129.77, 129.94 (aromatic CH), 130.43 (1-C of PMB ring), 137.43 (1-C of benzyl ring), 159.29, 159.42 (2 x 4-C of PMB rings), 169.91 (CH_3CO_2), 198.00 (6-C); *m/z* (FAB^-) 703 [$(M+NBA)^-$, 100%].

6.5 Synthesis of Intermediates and Analogues of Ins(1,4,5)P_3 Based on Adenophostin A

6.5.1 Methyl 3,4-di-*O*-benzoyl-2,6-di-*O*-benzyl-α-D-glucopyranoside (249)

The method employed is a modification of that described by Garegg *et al.*.[281] Hydrogen chloride in diethyl ether was added at room temperature under an atmosphere of nitrogen to a mixture of (**144**) (502mg, 1.1mmol), sodium cyanoborohydride (876mg, 13.9mmol), 3Å molecular sieves and dry THF (10cm^3) until the evolution of gas ceased. The system was stirred at room temperature for 20 min, when TLC (ethyl acetate : hexane 3:2) indicated complete consumption of starting material (R_f 0.66) to give a product (R_f 0.63). The suspension was diluted with ether (100cm^3) and filtered. The filtrate was washed with water (100cm^3) and saturated aqueous $NaHCO_3$ solution (100cm^3), dried ($MgSO_4$), filtered and concentrated to give a pale yellow oil, which was subjected to flash chromatography (eluent hexane : ethyl acetate 7:3). The product was dissolved in dry pyridine (5cm^3) and stirred with DMAP (25mg) and benzoyl chloride (0.25cm^3, 2.2mmol) for 1 h. Methanol (1cm^3) was added and stirring was continued for 15 min. The solvents were evaporated and the residue was extracted with ether (100cm^3). The organic extract was washed with 5M aqueous HCl (50cm^3), saturated aqueous $NaHCO_3$ solution (50cm^3) and saturated aqueous NaCl (50cm^3), dried ($MgSO_4$), filtered and concentrated. The oil thus obtained was purified by flash chromatography (eluent hexane : ethyl acetate 4:1) to give (**249**) as a colourless oil (482mg, 79% from **144**); R_f 0.74 (ether); m.p. 123–123.5°C (ethyl acetate-hexane); $[\alpha]_D$ –27.5 (*c* 3.8, $CHCl_3$); (Found: C, 71.8; H, 5.66. Calc. for $C_{35}H_{34}O_8$: C, 72.14; H, 5.89%); δ_H ($CDCl_3$; 270MHz) 3.47 (3 H, s, OCH_3), 3.55 (1 H, ABX, $^2J_{AB}$ 13.7, ^3J 4.4, 6-H), 3.61 (1 H, dd, $^2J_{AB}$ 13.7, ^3J 2.8, 6-H'), 3.78 (1 H, dd, *J* 3.5, 9.9, 2-H), 4.12 (1 H, ddd, *J* 2.8, 4.4, 9.8, 5-H), 4.48, 4.54 (2 H, AB, J_{AB} 12.1, PhCH_2O), 4.60, 4.62 (2 H, AB, J_{AB} 12.0, PhCH_2O), 4.80 (1 H, d, *J* 3.5, 1-H), 5.49 (1 H, t, *J* 9.9, 3-H), 5.92 (1 H, t, *J* 9.9, 4-H), 7.15–7.52 (16 H, m, aromatic CH), 7.86–7.94 (4 H, m, 2-H and 6-H of benzoyl rings); δ_C ($CDCl_3$; 67.8MHz) 55.43 (OCH_3), 68.41 (6-C), 68.63, 69.54, 72.14 (3-C, 4-C, 5-C), 72.95, 73.59 (2 x PhCH_2O), 76.81 (2-C), 97.89 (1-C), 127.50, 127.70, 127.86, 127.94, 128.18, 128.31, 129.91, 129.50 (aromatic CH), 129.67 (1-C of benzoyl

ring), 129.71 (aromatic CH), 129.77 (1-C of benzoyl ring), 132.82, 133.08 (aromatic CH), 137.49, 137.54 (2 x 1-C of benzyl rings), 165.32, 165.56 (2 x Ph*C*O_2); *m/z*: (FAB^+) 583 [$(M+1)^+$, 11%].

6.5.2 Acetolysis of (249)

The method employed is a modification of that described by Corrie.[216] A solution of (**249**) (480mg, 0.8mmol) in acetic anhydride (10cm^3) was stirred with freshly distilled, dry boron trifluoride etherate (0.1cm^3) at 0°C under an atmosphere of nitrogen for 20 min, when TLC (ether) indicated complete consumption of starting material (R_f 0.74) to give a minor product (R_f 0.68) and a major product (R_f 0.62). The reaction was quenched with saturated aqueous $NaHCO_3$ solution (5cm^3) and the mixture was extracted with ethyl acetate (200cm^3). The organic extract was washed with saturated aqueous $NaHCO_3$ solution (50cm^3) and water (50cm^3), dried ($MgSO_4$), filtered and concentrated. The pale yellow oil thus obtained was subjected to flash chromatography (eluent hexane : ethyl acetate 4:1) to give 1,6-di-*O*-acetyl-3,4-di-*O*-benzoyl-2-*O*-benzyl-D-glucopyranose (**251**) as a pale yellow oil which ran as a single spot on TLC (ether) but which was shown by ^{1}H NMR to be a *ca.* 3:1 α:β anomeric mixture (31mg, 7%); δ_H ($CDCl_3$; 270MHz) 2.02, 2.26 (6 H, 2s, 2 x CH_3CO_2), 3.87 (0.7 H, dd, *J* 3.7, 10.0, 2-H_α), 4.09–4.34 (3.3 H, m, 2-H_β, 5-H, 6-H, 6-H'), 4.51, 4.64 (2 H, AB, J_{AB} 12.2, PhCH_2O), 5.48 (1 H, t, *J* 9.7, 3-H), 5.82 (0.3 H, d, *J* 7.4, 1-H_β), 5.90 (1 H, t, *J* 9.8, 4-H), 6.48 (0.7 H, d, *J* 3.7, 1-H_α), 7.12–7.57 (11 H, m, aromatic CH), 7.85–7.97 (4 H, m, 2-H and 6-H of benzoyl rings). The α and β subscripts denote signals arising from the α- and β-anomers respectively.

Further elution gave methyl 6-*O*-acetyl-3,4-di-*O*-benzoyl-2-*O*-benzyl-α-D-glucopyranoside (**250**) as a colourless oil which spontaneously crystallised on standing (283mg, 64%); m.p. 76–78.5°C; $[\alpha]_D$ –23.5 (*c* 2.3, $CHCl_3$); (Found: C, 67.3; H, 5.56. Calc. for $C_{30}H_{30}O_9$: C, 67.39; H, 5.66%); δ_H ($CDCl_3$; 270MHz) 2.05 (3 H, s, CH_3CO_2), 3.48 (3 H, s, OCH_3), 3.77 (1 H, dd, *J* 3.7, 10.0, 2-H), 4.12–4.29 (3 H, m, 5-H, 6-H, 6-H'), 4.60, 4.63 (2 H, AB, J_{AB} 12.0, PhCH_2O), 4.79 (1 H, d, *J* 3.5, 1-H), 5.40 (1 H, t, *J* 9.7, 3-H), 5.94 (1 H, t, *J* 9.7, 4-H), 7.26–7.58 (11 H, m, aromatic CH), 7.80–7.98 (4 H, m, 2-H and 6-H of benzoyl rings); δ_C ($CDCl_3$; 67.8MHz) 20.71 (*C*H_3CO_2), 55.63

(OCH_3), 62.58 (6-C), 67.45, 69.49, 71.88 (3-C, 4-C, 5-C), 73.06 (Ph*C*H_2O),76.58 (2-C), 98.05 (1-C), 127.99, 128.28, 128.38, 128.44 (aromatic CH), 129.00 (1-C of benzoyl ring[s]), 129.74, 129.84, 132.98, 133.34 (aromatic CH), 137.49 (1-C of benzyl ring),165.48, 165.58 (2 x Ph*C*O_2),170.61 (CH_3*C*O_2); *m/z* (FAB$^+$) 535 [$(M+1)^+$, 6%].

6.5.3 Allyl α-D-glucopyranoside (254a)

The method employed is a modification of that described by Lee and Lee.[290] A mixture of D-glucose (18g, 0.1mole), Dowex 50X2-100 (10g) and allyl alcohol (200cm^3) was heated under reflux for 90 min. The suspension was cooled, filtered and the residue was well washed with ethanol (30cm^3). The combined filtrate and washings were concentrated and the syrupy residue was subjected to flash chromatography (loading solvent and eluent ethyl acetate : methanol 9:1). The eluate was concentrated to give a white solid (**254ab**), which was shown by ^{1}H NMR (D_2O; 270MHz; ref. int. HDO) to be a *ca.* 7:3 α:β anomeric ratio (1-H_α, δ 4.92, *J* 3.7; 1-H_β δ 4.46, *J* 7.9). Fractional crystallisation from ethyl acetate–ethanol (*ca.* 1:1) gave the pure α-anomer (**254a**) (3.1g, 14%); m.p. 99–100°C [lit.[288] 100.5–101.5°C]; $[\alpha]_D$ +140.6 (*c* 1.4, H_2O) [lit.[288] +151.1].

6.5.4 Allyl 2,6-di-*O*-benzoyl-α-D-glucopyranoside (261)

A mixture of (**254ab**) (5.0g, 22.7mmol) and dibutyltin oxide (5.9g, 23.8mmol) was heated under reflux in dry toluene (150cm^3) for 2h with continuous azeotropic removal of water (Dean-Stark trap). The solution was cooled to 0°C and triethylamine (1cm^3) and benzoyl chloride (5.5cm^3, 47.7mmol, dropwise) were added. The solution was allowed to warm to room temperature and was stirred for a further 16 h, when TLC (ethyl acetate) indicated a major product (R_f 0.63) and several minor products. Methanol (10cm^3) was added and stirring was continued for 5 min. The solvents were evaporated and the residue was taken up in ether (250cm^3). The solution was stirred with saturated aqueous $NaHCO_3$ solution (150cm^3) for 30 min and the resulting suspension was filtered through Celite. The ethereal layer was dried ($MgSO_4$), filtered and concentrated to give an orange oil, which was subjected to flash chromatography (eluent hexane : ethyl acetate 7:3). The eluate was concentrated and the residue was

crystallised from ethanol to give the title compound (3.4g, 35%); m.p. 135–136°C [lit.[291] 136–137°C]; $[\alpha]_D$ +70.1 (*c* 4.3, $CHCl_3$) [lit.[291] +74].

6.5.5 Allyl 2,6-di-*O*-benzoyl-3,4-*O*-isopropylidene-α-D-glucopyranoside (262)

A solution of (**261**) (1.2g, 2.8mmol) in acetone ($30cm^3$) was stirred with *p*-toluenesulphonic acid (30mg) and 2-methoxypropene ($1.5cm^3$, 15.7mmol) at room temperature for 5 min, when TLC (hexane : ethyl acetate 3:2) indicated complete conversion of starting material (R_f 0.13) to a product (R_f 0.48). The solvents were evaporated and the residue dissolved in ether ($50cm^3$). The organic extract was washed with saturated aqueous $NaHCO_3$ solution ($30cm^3$), dried ($MgSO_4$), filtered and concentrated. The residue was purified by flash chromatography (eluent hexane : ethyl acetate 19:1 then 17:3) to give the title compound as a pale yellow oil (774mg, 59%); $[\alpha]_D$ +69.4 (*c* 2.2, $CHCl_3$); (Found: C, 66.9; H, 6.20. Calc. for $C_{26}H_{28}O_8$: C, 66.64; H, 6.03%); δ_H ($CDCl_3$; 400MHz) 1.47, 1.50 (6 H, 2 s, 2 x isopropylidene CH_3), 3.54 (1 H, t, *J* 9.3, 4-H), 4.03 (1 H, m, C*H*HCH=CH_2), 4.19–4.32 (3 H, m, CH*H*CH=CH_2 , 3-H, 5-H), 4.48 (1 H, ABX, $^2J_{AB}$ 12.2, ^3J 6.3, 6-H), 4.68 (1 H, ABX, $^2J_{AB}$ 12.2, ^3J 2.4, 6-H'), 5.10 (1 H, m, ^3J 10.3, ^2J 1.0, $CH_2CH=CH_{cis}H_{trans}$), 5.19–5.25 (2 H, m, $CH_2CH=CH_{cis}H_{trans}$, 2-H), 5.36 (1 H, d, *J* 3.4, 1-H), 5.80 (1 H, m, $CH_2CH=CH_2$), 7.43–7.59 (6 H, m, aromatic CH), 8.06–8.11 (4 H, m, aromatic CH); δ_C ($CDCl_3$; 100MHz) 26.56, 26.82 (2 x isopropylidene CH_3), 64.01 (6-C), 69.12 ($CH_2CH=CH_2$), 70.01, 73.20, 74.76, 75.45 (4 x CH), 95.74 (1-C), 111.56 (C_q of isopropylidene), 117.85 ($CH_2CH=CH_2$), 128.38, 128.44 (aromatic CH), 129.48 (1-C of benzoyl phenyl ring), 129.68 (aromatic CH), 129.94 (1-C of benzoyl phenyl ring), 129.99 (aromatic CH), 133.16 (aromatic CH), 133.34 ($CH_2CH=CH_2$), 133.47 (aromatic CH), 165.95, 166.19 (2 x $PhCO_2$); *m/z* (FAB^+) 469 [$(M+1)^+$, 74%].

6.5.6 Allyl 3,4-*O*-isopropylidene-α-D-glucopyranoside (263)

Sodium hydroxide (514mg, 12.9mmol) was added to a solution of (**262**) (1.5g, 3.2mmol) in methanol ($100cm^3$) and the mixture was heated under reflux for 1 h. After cooling, carbon dioxide was bubbled through the cooled solution for 16 h. The solvent was evaporated and the glassy residue was extracted with chloroform (2 x $100cm^3$). The

combined organic extracts were washed with water (100cm^3), dried ($MgSO_4$), filtered and concentrated. The resultant residue was purified by flash chromatography (eluent hexane : ethyl acetate 7:3 then 1:1) to give the title compound as a colourless oil (779mg, 93%); R_f 0.35 (ethyl acetate); $[\alpha]_D$ +114.0 (*c* 1.3, $CHCl_3$); (Found: C, 55.2; H, 8.05. Calc. for $C_{12}H_{20}O_6$: C, 55.36; H, 7.75%); δ_H 1.45, 1.46 (6 H, 2 s, 2 x isopropylidene CH_3), 2.07 (1 H, br s, exch. D_2O, 6-OH), 2.40 (1 H, d, *J* 10.4, exch. D_2O, 2-OH), 3.30 (1 H, t, *J* 9.2, 4-H), 3.73–3.92 (5 H, m, 2-H, 3-H, 5-H, 6-H, 6-H'), 4.04–4.11 (1 H, m, C*H*HCH=CH_2), 4.23–4.30 (1 H, m, CH*H*CH=CH_2), 5.01 (1 H, d, *J* 3.8, 1-H), 5.25 (1 H, m, ^3J 10.3, ^2J 1.4, $CH_2CH=CH_{cis}H_{trans}$), 5.32 (1 H, m, ^3J 17.2, ^2J 1.5, $CH_2CH=CH_{cis}H_{trans}$), 5.92 (1 H, m, $CH_2CH=CH_2$); δ_C ($CDCl_3$; 67.8MHz) 26.42, 26.79 (2 x isopropylidene CH_3), 62.40 (6-C), 69.07 ($CH_2CH=CH_2$), 71.83, 72.39, 73.98, 78.75 (4 x CH), 97.97 (1-C), 111.12 (C_q of isopropylidene), 118.19 ($CH_2CH=CH_2$), 133.34 ($CH_2CH=CH_2$); *m/z* (FAB$^+$) 261 [(M+1)$^+$, 78%].

6.5.7 Allyl 2,6-di-*O*-benzyl-α-D-glucopyranoside (265)

A mixture of (**254a**) (2.0g, 9.1mmol) and dibutyltin oxide (5.7g, 22.7mmol) was heated under reflux in dry toluene (300cm^3) for 4 h with continuous azeotropic removal of water (Dean-Stark trap). The solution was cooled and concentrated to give a white residue which was dried on a vacuum line for 2 h. Benzyl bromide (18cm^3, 151.4mmol) was added and this mixture was stirred under an atmosphere of nitrogen at 100–110°C for 2 days. The pale yellow solution thus obtained was cooled, diluted with ether (100cm^3) and vigorously stirred with saturated aqueous $NaHCO_3$ solution (75cm^3) for 30 min. The resultant suspension was filtered through Celite and the organic layer was dried ($MgSO_4$), filtered and concentrated. Flash chromatography (eluent hexane : ethyl acetate 8:2 to remove benzyl bromide then 1:1) gave a mixture of benzylated products (2.8g, R_f 0.30–0.51 in ethyl acetate). Crystallisation from diisopropyl ether gave exclusively the title compound (1.6g, 44%); R_f 0.22 (ethyl acetate : hexane 3:2); m.p. 74–77°C; $[\alpha]_D$ +76.4 (*c* 0.8, $CHCl_3$); (Found: C, 68.8; H, 7.08. Calc. for $C_{23}H_{28}O_6$: C, 68.97; H, 7.05%); δ_H ($CDCl_3$; 270MHz) 3.12 (2 H, br s, exch. D_2O, 2 x OH), 3.37 (1 H, dd, *J* 3.7, 9.7, 2-H), 3.58 (1 H, t, *J* 9.2, 4-H), 3.68–3.78 (3 H, m, 5-H, 6-H, 6-H'), 3.88–3.98 (2 H, m, 3-H, C*H*HCH=CH_2), 4.10–4.15 (1 H, m, CH*H*CH=CH_2), 4.54, 4.59 (2 H,

AB, J_{AB} 12.2, PhCH_2O), 4.62, 4.67 (2 H, AB, J_{AB} 12.1, PhCH_2O), 4.81 (1 H, d, *J* 3.5, 1-H), 5.18 (1 H, m, ^3J 10.3, ^2J 1.0, $CH_2CH=CH_{cis}H_{trans}$), 5.29 (1 H, m, ^3J 17.2, ^2J 1.0, $CH_2CH=CH_{cis}H_{trans}$), 5.89 (1 H, m, $CH_2CH=CH_2$), 7.25–7.33 (10 H, m, aromatic CH); δ_C ($CDCl_3$; 67.8MHz) 68.29 ($CH_2CH=CH_2$), 69.44 (6-C), 70.03, 70.74, 72.72 (3 x CH), 72.75, 73.49 (2 x PhCH_2O), 79.08 (2-C), 95.46 (1-C), 117.87 ($CH_2CH=CH_2$), 127.58, 127.96, 128.05, 128.31, 128.46 (aromatic CH), 133.71 ($CH_2CH=CH_2$), 137.90, 137.96 (2 x 1-C of benzyl rings); *m/z*: (FAB$^-$) 553 [(M+NBA)$^-$, 100%]

A sample of (**265**) was converted to its 3,4-dibenzoate (**266**) with benzoyl chloride in pyridine in the usual way, R_f 0.72 (ethyl acetate : hexane 3:2); $[\alpha]_D$ –10.0 (*c* 1.4, $CHCl_3$); δ_H ($CDCl_3$; 400MHz) 3.57–3.61 (2 H, m, 6-H, 6-H'), 3.80 (1 H, dd, *J* 3.9, 10.1, 2-H), 4.08 (1 H, m, C*H*HCH=CH_2), 4.19 (1 H, ddd, *J* 5.7, 9.3, 9.8, 5-H), 4.25 (1 H, m, CH*H*CH=CH_2), 4.45–4.60 (4 H, m, 2 x PhCH_2O AB systems), 5.00 (1 H, d, *J* 3.4, 1-H), 5.25 (1 H, m, ^3J 10.3, ^2J 1.5, $CH_2CH=CH_{cis}H_{trans}$), 5.38 (1 H, m, ^3J 17.6, ^2J 1.5, $CH_2CH=CH_{cis}H_{trans}$), 5.52 (1 H, t, *J* 10.3, 4-H), 5.95–6.00 (2 H, m, t of 3-H overlapping with $CH_2CH=CH_2$), 7.14–8.13 (20 H, m, aromatic CH).

The mother liquor of the benzylation mixture was reacted with 2-methoxypropene and *p*-toluenesulphonic acid in acetone as described for (**262**). A single new product was formed by TLC (R_f 0.71 in ethyl acetate : hexane 3:2). Purification and flash chromatography as for (**262**) gave allyl 2,3-di-*O*-benzyl-4,6-*O*-isopropylidene-α-D-glucopyranoside (**267**) as a pale yellow oil (480mg, 12% from **254a**); $[\alpha]_D$ +26.5 (*c* 0.7, $CHCl_3$); (Found: C, 71.1; H, 7.40. Calc. for $C_{26}H_{32}O_6$: C, 70.89; H, 7.32%); δ_H ($CDCl_3$; 400MHz) 1.43, 1.48 (6 H, 2 s, 2 x isopropylidene CH_3), 3.50 (1 H, dd, *J* 3.9, 9.3, 2-H), 3.61–3.72 (3 H, m, 4-H, 5-H, 6-H_{ax}), 3.83 (1 H, dd, *J* 3.4, 8.3, 6-H_{eq}), 3.90 (1 H, *J* 9.3, 3-H), 3.97–4.03 (1 H, m, C*H*HCH=CH_2), 4.14–4.18 (1 H, m, CH*H*CH=CH_2), 4.65, 4.81 (2 H, AB, J_{AB} 12.2, PhCH_2O), 4.75 (1 H, d, *J* 3.4, 1-H), 4.80, 4.85 (2 H, AB, J_{AB} 11.7, PhCH_2O), 5.23 (1 H, m, ^3J 10.3, ^2J 1.0, $CH_2CH=CH_{cis}H_{trans}$), 5.32 (1 H, m, ^3J 17.1, ^2J 1.0, $CH_2CH=CH_{cis}H_{trans}$), 5.93 (1 H, m, $CH_2CH=CH_2$), 7.24–7.39 (10 H, m, aromatic CH); δ_C ($CDCl_3$; 67.8MHz) 19.18 (axial CH_3 of isopropylidene), 29.22 (equatorial CH_3 of isopropylidene), 62.58 (6-C), 63.37 (CH), 68.36 ($CH_2CH=CH_2$), 73.57 (PhCH_2O), 74.91 (CH), 75.07 (PhCH_2O), 78.99, 79.11 (2-C, 3-C), 96.78 (1-C), 99.34 (C_q of

isopropylidene), 118.23 ($CH_2CH=CH_2$), 127.43, 127.78, 127.85, 128.02, 128.22, 128.36 (aromatic CH), 133.69 ($CH_2CH=CH_2$), 138.29, 139.08 (2 x 1-C of benzyl rings); *m/z* (FAB^+) 441 [$(M+1)^+$, 40%].

6.5.8 Allyl 2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-α-D-glucopyranoside (264)

a) A solution of (**263**) (717mg, 2.8mmol) in dry DMF (5cm³) was stirred with sodium hydride (215mg of an 80%$^{w}/_{w}$ dispersion in mineral oil, 6.9mmol) and benzyl bromide (0.7cm³, 6.1mmol) at room temperature for 3 h. Methanol (5cm³) was added and stirring was continued for 15 min. The solvents were evaporated and the residue was dissolved in ether (100cm³). The ethereal extract was washed with water (50cm³), dried ($MgSO_4$), filtered and concentrated. The residual oil was purified by flash chromatography (eluent hexane : ethyl acetate 19:1) to give the title compound as a pale yellow oil (922mg, 76%); R_f 0.68 (hexane:ethyl acetate 2:3); $[\alpha]_D$ +27.8 (*c* 2.0, $CHCl_3$); (Found: C, 70.9; H, 7.38. Calc. for $C_{26}H_{32}O_6$: C, 70.89; H, 7.32%); δ_H ($CDCl_3$; 400MHz) 1.45, 1.47 (6 H, 2 s, 2 x isopropylidene CH_3), 3.34 (1 H, t, *J* 9.8, 4-H), 3.61 (1 H, ABX, $^2J_{AB}$ 10.7, ^3J 5.4, 6-H), 3.70 (1 H, dd, *J* 2.4, 10.3, 2-H), 3.74 (1 H, ABX, $^2J_{AB}$ 10.7, ^3J 2.4, 6-H'), 3.97–4.03 (3 H, m, 3-H, 5-H, C*H*HCH=CH_2), 4.13–4.21 (1 H, m, CH*H*CH=CH_2), 4.59, 4.60 (2 H, AB, J_{AB} 12.7, PhCH_2O), 4.63, 4.84 (2 H, AB, J_{AB} 12.2, PhCH_2O), 4.92 (1 H, d, *J* 3.4, 1-H), 5.19 (1 H, m, ^3J 10.7, ^2J 1.4, $CH_2CH=CH_{cis}H_{trans}$), 5.33 (1 H, m, ^3J 17.1, ^2J 1.5, $CH_2CH=CH_{cis}H_{trans}$), 5.92 (1 H, m, $CH_2CH=CH_2$), 7.25–7.58 (10 H, m, aromatic CH); δ_C ($CDCl_3$; 100MHz) 26.54, 26.92 (2 x isopropylidene CH_3), 68.65 ($CH_2CH=CH_2$), 69.42 (6-C), 71.01 (CH), 71.96, 73.41 (2 x PhCH_2O), 74.32, 77.60, 77.83 (3 x CH), 96.80 (1-C), 110.75 (C_q of isopropylidene), 117.90 ($CH_2CH=CH_2$), 127.47, 127.56, 127.74, 128.02, 128.35 (aromatic CH), 133.83 ($CH_2CH=CH_2$), 138.11 (1-C of benzyl ring[s]); *m/z*: (FAB^+) 441 [$(M+1)^+$, 12%].

b) Using the method for the preparation of (**262**) from (**261**), the title compound was prepared in 71% yield from (**265**).

6.5.9 Allyl 2,6-di-*O*-benzyl-3,4-di-*O*-(*p*-methoxybenzyl)-α-D-glucopyranoside (257)

A solution of (**265**) (1.9g, 4.7mmol) in dry DMF (10cm^3) was stirred with sodium hydride (326mg of an 80%$^{w}/_{w}$ dispersion in mineral oil, 10.4mmol) and *p*-methoxybenzyl chloride (1.4cm^3, 10.0mmol) at room temperature for 3 h, when TLC (ethyl acetate : hexane 3:2) indicated consumption of starting material (R_f 0.27) to give a product (R_f 0.71). Methanol (5cm^3) was added and stirring was continued for 15 min. The solvents were evaporated and the residue was dissolved in chloroform (200cm^3). The organic solution was washed with water (100cm^3), dried ($MgSO_4$), filtered and concentrated. The residual oil was purified by flash chromatography (eluent hexane : ethyl acetate 19:1) to give the title compound as a pale yellow oil (2.2g, 72%); $[\alpha]_D$ +13.8 (*c* 1.9, $CHCl_3$); (Found: C, 73.4; H, 6.88. Calc. for $C_{39}H_{44}O_8$: C, 73.09; H, 6.93%); δ_H ($CDCl_3$; 400MHz) 3.55 (1 H, dd, *J* 3.7, 9.7, 2-H), 3.57–3.64 (2 H, m, 4-H, 6-H), 3.68–3.80 (2 H, m, 5-H, 6-H'), 3.77, 3.80 (6 H, 2 s, 2 x OCH_3), 3.95–4.18 (3 H, m, 3-H, $CH_2CH{=}CH_2$), 4.36–4.94 (9 H, m, 4 x $ArCH_2O$ AB systems, 1-H), 5.19 (1 H, m, ^3J 10.4, ^2J 0.9, $CH_2CH{=}CH_{cis}H_{trans}$), 5.29 (1 H, m, ^3J 17.2, ^2J 1.0, $CH_2CH{=}CH_{cis}H_{trans}$), 5.90 (1 H, m, $CH_2CH{=}CH_2$), 6.78–6.88 (4 H, m, 2 x 3-H and 5-H of PMB rings), 7.04 (2 H, d, *J* 8.5, 2-H and 6-H of PMB ring), 7.24–7.38 (12 H, m, aromatic CH); δ_C ($CDCl_3$; 67.8MHz) 55.24 (OCH_3), 68.15, 68.45 (6-C and $CH_2CH{=}CH_2$), 70.27 (5-C), 73.17, 73.40, 74.63, 75.36 (4 x $ArCH_2O$), 77.39, 79.94, 81.88 (2-C, 3-C, 4-C)), 95.72 (1-C), 113.75, 113.78 (3-C and 5-C of PMB rings), 118.06 ($CH_2CH{=}CH_2$), 127.60, 127.78, 127.83, 128.02, 128.31, 128.36, 129.48, 129.53 (aromatic CH), 131.13 (1-C of PMB ring[s]), 133.79 ($CH_2CH{=}CH_2$), 137.99, 138.27 (2 x 1-C of benzyl rings), 159.19, 159.14 (2 x 4-C of PMB rings); *m/z*: (FAB$^+$) 641 [(M+1)$^+$, 10%].

6.5.10 (*cis*-Prop-1-enyl) 2,6-di-*O*-benzyl-3,4-di-*O*-(*p*-methoxybenzyl)-α-D-gluco-pyranoside (269a)

A solution of (**257**) (2.0g, 3.1mmol), and freshly sublimed potassium *t*-butoxide (1.8g, 15.6mmol) in dry DMSO (20cm^3) was stirred at 50°C under an atmosphere of nitrogen for 3 h, when TLC ($CHCl_3$: acetone 10:1) indicated total consumption of starting material (R_f 0.66) to give a product (R_f 0.71). The dark brown solution was cooled, water (20cm^3) was added and the system was extracted with ether (3 x 100cm^3). The

combined organic extracts were washed with saturated aqueous KCl solution (2 x 150cm^3), dried ($MgSO_4$), filtered and concentrated. The pale yellow oil thus obtained was purified by flash chromatography (eluent hexane : ethyl acetate 4:1) to give a mixture of *trans* and *cis*-prop-1-enyl isomers (**269ab**) (1.7g, 85%). A portion was recolumned twice with the same solvent system to provide an analytical sample of the pure *cis* isomer (**269a**) as a pale yellow oil; $[\alpha]_D$ +2.9 (*c* 1.4, $CHCl_3$); (Found: C, 73.2; H, 6.89. Calc. for $C_{39}H_{44}O_8$: C, 73.09; H, 6.93%); δ_H ($CDCl_3$; 270MHz) 1.63 (3 H, dd, ^4J 1.5, ^3J 6.8, CH=CHCH_3), 3.56–3.80 (5 H, m, 2-H, 4-H, 5-H, 6-H, 6-H'), 3.77, 3.80 (6 H, 2 s, 2 x OCH_3), 4.02 (1 H, t, *J* 9.2, 3-H), 4.39–4.94 (10 H, m, 4 x ArCH_2O AB systems, 1-H, CH=C*H*CH_3), 6.00 (1 H, dd, ^4J 1.5, ^3J 3.6, C*H*=$CHCH_3$), 6.78–6.89 (4 H, m, 2 x 3-H and 5-H of PMB rings), 7.05 (2 H, d, *J* 8.6, 2-H and 6-H of PMB ring), 7.24–7.34 (12 H, m, aromatic CH); δ_C ($CDCl_3$; 100MHz) 9.62 (CH=CH*C*H_3), 55.27 (OCH_3), 68.16 (6-C), 70.75 (5-C), 73.23, 73.37, 74.78, 75.40 (4 x Ar*C*H_2O), 77.12, 79.61, 81.73 (2-C, 3-C, 4-C), 97.46 (1-C), 104.46 (CH=*C*HCH_3), 113.81 (3-C and 5-C of PMB ring[s]), 127.69, 127.87, 127.96, 128.35, 128.46, 129.52, 129.59, 129.63 (aromatic CH), 130.39, 131.03 (2 x 1-C of PMB rings), 137.89, 138.24 (2 x 1-C of benzyl rings), 142.21 (*C*H=$CHCH_3$), 159.18, 159.25 (2 x 4-C of PMB rings); *m/z*: (FAB$^+$) 641 [$(M+1)^+$, 20%].

6.5.11 2,6-Di-*O*-benzyl-3,4-di-*O*-(*p*-methoxybenzyl)-D-glucopyranose (240)

A solution of (**269ab**) (1.5g, 2.3mmol) in acetone (50cm^3) was heated to 50°C (oil bath). Aqueous 1M HCl (5cm^3) was added and stirring was continued for 20 min, when TLC (chloroform : acetone 10:1) indicated total consumption of starting material (R_f 0.70) to give a product (R_f 0.28). Solid $NaHCO_3$ (2g) was added and stirring was continued as the suspension was allowed to cool to room temperature. The solvents were evaporated and the residue was extracted with ether (3 x 100cm^3). The combined organic extracts were washed with saturated aqueous $NaHCO_3$ solution (150cm^3) and water (150cm^3), dried ($MgSO_4$), filtered and concentrated. Recrystallisation from a minimum of ether gave the title compound (1.1g, 77%), which reduced Fehling's solution; m.p. 120–131°C; $[\alpha]_D$ +5.1 (*c* 1.4, $CHCl_3$, 2 h); (Found: C, 71.8; H, 6.66. Calc. for $C_{36}H_{40}O_8$: C, 71.97; H, 6.72%); δ_H ($CDCl_3$; 270MHz) 1.64 (0.5 H, s, exch. D_2O, OH

β), 3.09 (0.5 H, d, *J* 2.6, exch. D_2O, OH_α), 3.37 (0.5 H, dd, *J* 7.6, 9.9, 2-H_β), 3.48–3.80 (4.5 H, m, 2-H_α, 3-H_α or 3-H_β, 5-H_α or 5-H_β, 4-H, 6-H, 6-H'), 3.78, 3.80 (6 H, 2 s, 2 x OCH_3), 3.94 (0.5 H, t, *J* 9.2, 3-H_α or 3-H_β), 4.01 (0.5 H, ddd overlapping with t at 3.94ppm, 5-H_α or 5-H_β), 4.39–4.93 (8.5 H, 1-H_β, 4 x Ar*C*H_2O AB systems), 5.21 (0.5 H, t, *J* 2.6, simplifies to d on D_2O exch., 1-H_α), 6.79–6.88 (4 H, m, 2 x 3-H and 5-H of PMB rings), 7.02–7.07 (2 H, m, 2-H and 6-H of PMB ring), 7.21–7.34 (12 H, m, aromatic CH). The α and β subscripts denote signals arising from the α- and β-anomers respectively; *m/z*: (FAB$^-$) 753 [(M+NBA)$^-$, 100%].

6.5.12 (2-Hydroxyethyl) 2,6-di-*O*-benzyl-α-D-glucopyranoside (270)

The method employed is a modification of that described by Ferrier and Stütz.[185] A saturated aqueous solution of sodium metaperiodate (20cm^3) containing osmium tetraoxide (80mg, 0.3mmol) was added to a solution of (**264**) (392mg, 0.9mmol) in ether (20cm^3) and the mixture was vigorously stirred at room temperature for 5 h, when TLC (ethyl acetate : hexane 2:3) indicated consumption of starting material (R_f 0.56) to give a product (R_f 0.08–0.18). The mixture was diluted with ether (100cm^3) and water (50cm^3) and the organic layer was dried ($MgSO_4$), filtered and concentrated to give a dark grey residue which was dissolved in methanol (30cm^3) and cooled to 0°C. Sodium borohydride (480mg, 12.7mmol) was added and the suspension was stirred for 1 h. The mixture was concentrated and the residue partitioned between ether (100cm^3) and water (50cm^3). The organic layer was dried ($MgSO_4$), filtered and concentrated. Purification by flash chromatography (eluent hexane : ethyl acetate 1:1 then ethyl acetate) gave the title compound as a colourless oil (203mg, 56%); R_f 0.52 (ethyl acetate : MeOH 4:1); [α]$_D$ +48.2 (*c* 2.6, $CHCl_3$); (Found: C, 65.6; H, 7.12. Calc. for $C_{22}H_{28}O_7$: C, 65.32; H, 6.98%); δ_H (400MHz; $CDCl_3$) 3.35 (1 H, dd, *J* 3.4, 9.8, 2-H), 3.38-3.43 (1 H, m, -C*H*HCH$_2$-), 3.45 (1 H, t, *J* 9.3, 4-H), 3.58-3.69 (6 H, m, simplifies to 5 H, m, on D_2O exch, OH, 6-H, 6-H', -CH*HC*H_2-), 3.74 (1 H, ddd, *J* 2.4, 4.9, 9.8, 5-H), 3.83 (2 H, br s, exch D_2O, 2 x OH), 3.97 (1 H, t, *J* 9.8, 3-H), 4.50, 4.55 (2 H, AB, J_{AB} 12.2, PhCH_2O), 4.56 (1 H, AB, J_{AB} 12.2, PhC*H*HO), 4.66 (1 H, d, *J* 3.9, 1-H), 4.73 (1 H, AB, J_{AB} 12.2, PhCH*H*O), 7.23-7.36 (10 H, m, aromatic CH); δ_C (100MHz; $CDCl_3$) 61.47 (2'-C), 69.37 (6-C), 70.28 (1'-C), 70.50, 70.63, 72.88 (3-C, 4-C, 5-C), 73.55 (2 x Ph*C*H$_2$O), 79.52 (2-

C), 97.15 (1-C), 127.67, 127.71, 128.33, 128.38, 128.44, 128.66 (aromatic CH), 137.54, 138.00 (2 x 1-C of benzyl rings); *m/z* (FAB$^-$) 403 $(M-1)^-$, 50%; 557 $(M+NBA)^-$, 100%.

6.5.13 (2-Hydroxyethyl) 2,6-di-*O*-benzyl-2',3,4-tris(dibenzylphospho)-α-D-glucopyranoside (271)

A mixture of bis(benzyloxy)(diisopropylamino)phosphine (565mg, 1.6mmol), dry dichloromethane (3cm^3) and 1 *H*-tetrazole (220mg, 3.1mmol) was stirred at room temperature for 15 min, whereupon a solution of (**270**) (106mg, 0.26mmol) in dry dichloromethane (2cm^3) was added and stirring was continued for a further 30 min. TLC (ethyl acetate) indicated complete conversion of starting material (R_f 0.17) to a product (R_f 0.76 with streaking) and ^{31}P NMR spectroscopy showed phosphite triester signals at 140.72, 141.31 (AB, J_{AB} 4.9) and 139.05ppm. The system was cooled to –78°C, *m*-chlorperoxybenzoic acid (900mg) was added and the system was warmed to room temperature. After 10 min TLC showed conversion of the trisphosphite to a new product (R_f 0.46). The mixture was extracted with ethyl acetate (100cm^3) and the organic extract was washed with 50cm^3 each of 10%^{w}/$_{v}$ aqueous Na_2SO_3 solution, 1M HCl, saturated aqueous $NaHCO_3$ solution and saturated aqueous NaCl solution. The organic solution was dried ($MgSO_4$), filtered and concentrated to give a pale yellow solid, which was purified by flash chromatography (eluent $CHCl_3$: acetone 20:1 then 10:1) to give the title compound as a colourless oil (247mg, 80%); $[\alpha]_D$ +12.5 (*c* 1.2, $CHCl_3$); (Found: C, 65.1; H, 5.62. Calc. for $C_{64}H_{67}O_{16}P_3$: C, 64.84; H, 5.70%); δ_H ($CDCl_3$; 400MHz) 3.45–3.51 (1 H, m, OC*H*HCH$_2$OP[O][OBn]$_2$), 3.56 (1 H, dd, *J* 3.4, 9.3, 2-H), 3.65–3.70 (2 H, m, 6-H, OCH*H*CH$_2$OP[O][OBn]$_2$), 3.73–3.77 (1 H, ABX, $^2J_{AB}$ 10.7, ^3J 3.9, 6-H'), 3.89 (1 H, ddd, *J* 3.9, 9.3, 9.8, 5-H), 4.09–4.17 (2 H, m, OCH$_2$C*H*$_2$OP[O][OBn]$_2$), 4.32, 4.45 (2 H, AB, J_{AB} 11.7, PhC*H*$_2$O), 4.43–4.48 (1 H, AB, PhC*H*HO), 4.61–4.68 (3 H, m, PhCH*H*O, 3-H, 4-H), 4.86–5.07 (13 H, m, 6 x PhC*H*$_2$O AB systems, 1-H), 7.12–7.32 (40 H, m, aromatic CH); δ_C ($CDCl_3$; 100MHz) 66.10, 66.95 (2'-C[with C-P coupling]), 68.00 (6-C or 1'-C), 66.94, 69.15 (PO*C*H$_2$Ph [with C-P coupling]), 69.35, 69.40, 69.73 (6 x PO*C*H$_2$Ph), 73.08, 73.21 (2 x Ph*C*H$_2$O), 74.40, 77.91, 78.42, 78.46 (4 x CH), 96.69 (1-C), 127.50, 127.61, 127.74, 127.85, 127.91, 128.00, 128.03, 128.24, 128.31, 128.40, 128.47, 128.58 (aromatic CH), 135.72, 135.79, 135.86, 135.92, 136.15, 136.23 (6 x 1-C of benzylphospho rings), 137.95, 138.07 (2 x 1-

C of benzyl rings); δ_P ($CDCl_3$; 162MHz) –2.29 (sextet, J_{HP} 8.0), –1.85 (sextet, J_{HP} 7.6), –0.89 (septet, J_{HP} 7.9, OCH_2CH_2OP[O][OBn]$_2$); *m/z*: (FAB$^+$) 641 [(M+1)$^+$, 10%].

6.5.14 (2-Hydroxyethyl) α-D-glucopyranoside 2',3,4-trisphosphate (235)

Ammonia was condensed into a three-necked flask at –78°C. An excess of sodium was added to dry the liquid ammonia, about 30cm^3 of which was then distilled into a second three-necked flask and kept at –78°C. Sodium was added until the solution remained blue for 10 min. A solution of (**271**) (106mg) in dry dioxane (2cm^3) was added. The mixture was stirred for 90 sec, then the reaction was quenched with methanol (1cm^3), followed by water (1cm^3). The solvents were evaporated. The residue was dissolved in de-ionised water (300cm^3) and purified by ion-exchange chromatography on Q Sepharose fast flow, eluting with a gradient of triethylammonium bicarbonate buffer (0–1M), pH 8.0 . The triethylammonium salt of (**235**) eluted between 470–550 mM buffer; $[\alpha]_D$ +90.5 (*c* 0.8 calc. for free acid, TEAB, pH 8.6); δ_H (D_2O, pH *ca.* 4; 400MHz) 3.54-3.60 (1 H, m, 5-H), 3.55 (1 H, dd, *J* 3.9, 9.8, 2-H), 3.67-3.74 (4 H, m, $OCH_2CH_2OPO_3^{2-}$, 6-H, 6-H'), 3.82-3.91 (3 H, m, 3-H or 4-H, $OCH_2CH_2OPO_3^{2-}$), 4.26 (1 H, ddd, *J* 8.8, 9.3, 3-H or 4-H), 4.85 (1 H, d, *J* 3.9, 1-H); δ_P (D_2O, pH *ca.* 4; 36MHz) 0.32, 0.45, 0.52 (3 s); *m/z*: (FAB$^-$) 463 [(M-1)$^-$, 100%]; (Found: M$^-$, 462.983. Calc. for $C_8H_{18}O_{16}P_3$ [M-H]$^-$ 462.981).

6.5.15 Allyl 2,6-di-*O*-benzyl-3,4-bis(di-*p*-chlorobenzylphospho)-α-D-gluco-pyranoside (278)

A mixture of bis(*p*-chlorobenzyloxy)(diisopropylamino)phosphine[242] (8.8g, 21.3mmol), dry dichloromethane (10cm^3) and 1*H*-tetrazole (2.2g, 32.0mmol) was stirred at room temperature for 20 min, whereupon (**265**) (2.1g, 5.3mmol) was added and stirring was continued for a further 40 min. ^{31}P NMR spectroscopy showed a bisphosphite triester AB system at δ_P 141.55 and 140.93ppm (*J* 5.5Hz), and excess tetrazolide at 126.26ppm. The system was cooled to –78°C and MCPBA (7.3g) was added. Stirring was continued for 10 min, when ^{31}P NMR spectroscopy revealed consumption of phosphites to give a new product at δ_P *ca.* –2ppm. The mixture was extracted with ether (200cm^3) and the organic extract was washed with 100cm^3 each of 10%$^{w}/_{v}$ aqueous Na_2SO_3 solution, 1M

HCl, saturated aqueous $NaHCO_3$ solution and saturated aqueous NaCl solution. The organic solution was dried ($MgSO_4$), filtered and concentrated to give a waxy solid which was purified by flash chromatography (eluent chloroform : acetone 20:1) to give (**278**) as a colourless oil (4.7g, 85%); R_f 0.56 (chloroform : acetone 10:1); $[\alpha]_D$ +15.2 (*c* 2.6, $CHCl_3$); δ_H ($CDCl_3$; 400MHz) 3.58 (1 H, dd, *J* 3.4, 9.8, 2-H), 3.70 (1 H, ABX, $^2J_{AB}$ 11.0, ^3J 1.8, 6-H), 3.77 (1 H, ABX, $^2J_{AB}$ 11.0, ^3J 4.0, 6-H'), 3.87–3.93 (2 H, m, 5-H, C*H*HCH=CH_2), 4.10–4.15 (1 H, m, CH*H*CH=CH_2), 4.42, 4.50 (2 H, AB, J_{AB} 11.9, PhCH_2O), 4.52, 4.67 (2 H, AB, J_{AB} 12.0, PhCH_2O), 4.78 (1 H, d, *J* 3.4, 1-H), 4.82–5.00 (10 H, m, 4 x ArCH_2O AB systems, 3-H, 4-H), 5.21 (1 H, m, ^3J 10.4, ^2J 0.9, CH_2CH=C$H_{cis}H_{trans}$), 5.31 (1 H, m, ^3J 17.1, CH_2CH=$CH_{cis}H_{trans}$), 5.89 (1 H, m, CH_2CH=CH_2), 7.05–7.34 (26 H, m, aromatic CH); δ_C ($CDCl_3$; 100MHz) 68.03 (CH_2CH=CH_2), 68.43, 68.67, 68.94 (P[O]OCH_2Ar with CP coupling, *J*=3–6Hz), 69.20 (CH), 72.95, 73.30 (2 x PhCH_2O), 74.63, 77.87, 78.63 (3 x CH), 95.21 (1-C), 118.27 (CH_2CH=CH_2), 127.52, 128.00, 128.29, 128.44, 128.55, 128.62, 128.71, 129.06, 129.15, 129.26, 129.33 (aromatic CH), 133.34 (CH_2CH=CH_2), 134.12, 134.20, 134.34, 134.49 (4 x 1-C of *p*-chlorobenzylphospho rings), 137.65, 137.98 (2 x 1-C of benzyl rings); δ_P ($CDCl_3$; 162MHz) (^{1}H-coupled) –2.38 (sextet, J_{HP} 8.2), –2.00 (sextet J_{HP} 7.8); *m/z* (FAB^+) 1057 (66%), 1059 (90%), 1061 (52%), 1063 (16%) [all $(M+1)^+$].

6.5.16 (2-Hydroxyethyl) 2,6-di-*O*-benzyl-3,4-bis(di-*p*-chlorobenzylphospho)-α-D-glucopyranoside (279)

To a saturated aqueous solution of sodium metaperiodate (50cm^3) containing osmium tetraoxide (100mg, 0.4mmol) was added a solution of (**278**) (2.0g, 1.9mmol) in ether (50cm^3). The mixture was stirred at room temperature for 20 h, when TLC (chloroform : acetone 10:1) indicated consumption of starting material (R_f 0.56) to give a product (R_f 0.05–0.15). The mixture was diluted with ether (100cm^3) and the organic layer was washed with saturated aqueous NaCl solution (50cm^3), dried ($MgSO_4$), filtered and concentrated. The grey residue thus obtained was subjected to flash chromatography (eluent chloroform : acetone 10:1). The product was dissolved in methanol (50cm^3) and this solution was stirred with sodium borohydride (176mg, 4.7mmol) at room temperature for 30 min. The solvent was evaporated and the residue extracted with ether (200cm^3). The organic extract was washed with 50cm^3 each of 1M aqueous HCl,

water and saturated aqueous NaCl solution, dried ($MgSO_4$), filtered and concentrated. The residue was purified by flash chromatography (eluent chloroform : acetone 9:1) to give (**279**) as a colourless oil (922mg, 46%); $[\alpha]_D$ +17.3 (*c* 1.3, $CHCl_3$); (Found: C, 56.4; H, 4.81. Calc. for $C_{50}H_{50}Cl_4O_{13}P_2$: C, 56.60; H, 4.75%); δ_H ($CDCl_3$; 400MHz) 1.90–2.40 (1 H, br s, exch. D_2O, OH), 3.48–3.52 (1 H, m, C*H*HCH_2), 3.57 (1 H, dd, *J* 3.4, 9.8, 2-H), 3.66–3.74 (5 H, m, CH*HC*H_2, 6-H, 6-H'), 4.00 (1 H, ddd, *J* 2.9, 9.8, 9.9, 5-H), 4.41–4.58 (4 H, m, 3 x PhC*H*H AB protons, 4-H), 4.65–4.70 (2 H, m, PhC*H*H, 1-H), 4.83–5.01 (9 H, m, 4 x ArCH_2O AB systems, 3-H), 7.05–7.32 (26 H, m, aromatic CH); δ_C ($CDCl_3$; 100MHz) 61.54 (2'-C), 68.11, 68.51, 68.64, 68.75, 68.95, 69.26 (4 x P[O]OCH_2Ar with CP coupling, *J*=3–6Hz, 1'-C, 6-C), 71.01 (CH), 73.35, 73.39 (2 x PhCH_2O), 74.78, 78.09, 78.47 (3 x CH), 96.89 (1-C), 127.63, 127.71, 128.05, 128.22, 128.36, 128.60, 128.64, 128.77, 129.11, 129.30, 129.39 (aromatic CH), 134.25, 134.34, 134.42, 134.60 (4 x 1-C of *p*-chlorobenzylphospho rings) 137.47, 137.73 (2 x 1-C of benzyl rings); δ_P ($CDCl_3$; 109MHz) (^{1}H-coupled) –2.00 (sextet J_{HP} 6.8), –1.47 (sextet, J_{HP} 10.0); *m/z* (FAB^+) 1061(45%), 1063 (50%), 1065 (30%) [all $(M+1)^+$].

6.5.17 (2-Hydroxyethyl) 2,6-di-*O*-benzyl-3,4-bis(di-*p*-chlorobenzylphospho)-2'-(di-*p*-chlorobenzylphosphorothio)-α-D-glucopyranoside (280)

The method employed is a modification of that described by Mills *et al.*[101] A mixture of bis(*p*-chlorobenzyloxy)(diisopropylamino)phosphine[242] (454mg, 1.1mmol), dry dichloromethane (5cm^3) and 1*H*-tetrazole (117mg, 1.7mmol) was stirred at room temperature for 15 min, whereupon a solution of (**279**) (590mg, 0.6mmol) in dry dichloromethane (5cm^3) was added and stirring was continued for a further 30 min. ^{31}P NMR spectroscopy showed a phosphite peak at δ_P 139.45ppm and phosphate peaks at δ_P –1.82 and –2.35ppm. The solvents were evaporated and the residue was redissolved in dry DMF-dry pyridine 2:1 (3cm^3). Sulphur (28mg, 0.9mmol) was added and the system was stirred for 30 min, when ^{31}P NMR spectroscopy indicated major peaks at δ_P *ca.* 67.8, –2.2 and –2.6ppm. The suspension was filtered and the solvents were evaporated from the filtrate. The residue was extracted with dichloromethane (100cm^3) and the organic extract was washed with 50cm^3 each of 0.1M aqueous HCl, 5%$^{w}/_{v}$ aqueous $NaHCO_3$ solution and water, dried ($MgSO_4$), filtered and concentrated. The residue was

purified by flash chromatography (eluent chloroform : acetone 10:1) to give (**280**) as a colourless oil (509mg, 61%); $[\alpha]_D$ +19.7 (*c* 7.5, $CHCl_3$); δ_H ($CDCl_3$; 400MHz) 3.03–3.77 (5 H, m, 2-H, 6-H, 6-H', $OCH_2CH_2OP[S][OPCB]_2$), 3.93 (1 H, ddd, *J* 3.4, 11.9, 5-H), 4.14–4.19 (2 H, m, $OCH_2CH_2OP[S][OPCB]_2$), 4.36–5.02 (19 H, m, 1-H, 3-H, 4-H, 8 x $ArCH_2O$ AB systems), 7.01–7.32 (34 H, m, aromatic CH); δ_C ($CDCl_3$; 100MHz) 66.73 (2'-C [with C-P coupling]), 67.92 (1'-C or 6-C), 68.63, 68.96, 69.02 ($POCH_2Ar$ [with C-P coupling, *J*=3–7Hz], 1'-C or 6-C), 69.23 (CH), 72.93, 73.28 (2 x $PhCH_2O$), 74.41, 77.27, 77.83, (3 x CH), 96.40 (1-C), 127.50, 127.58, 127.94, 128.03, 128.31, 128.47, 128.53, 128.60, 128.71, 128.80, 129.00, 129.11, 129.24, 129.33, 129.44, 129.48 (aromatic CH), 134.03, 134.14, 134.34, 134.38, 134.42, 134.53 (6 x 1-C of *p*-chlorobenzylphospho rings), 137.60, 137.89 (2 x 1-C of benzyl rings); δ_P ($CDCl_3$; 162MHz) –2.33 (sextet, J_{HP} 8, ring phosphate), –1.89 (sextet, J_{HP} 7.7, ring phosphate), 68.76 (septet, J_{HP} 10, $P[S][OPCB]_2$); *m/z* (FAB^+) 1406 (47%), 1408 (75%), 1410 (57%), 1412 (17%), 1414 (9%) [all $(M+1)^+$].

6.5.18 (2-Hydroxyethyl) α-D-glucopyranoside 3,4-bisphosphate-2'-phosphorothioate (276)

Ammonia was condensed into a three-necked flask at –78°C. An excess of sodium was added to dry the liquid ammonia, about $30cm^3$ which was then distilled into a second three-necked flask and kept at –78°C. Sodium was added until the solution remained blue for 10 min. A solution of (**280**) (125mg, 89μmol) in dry dioxane ($2cm^3$) was added. The mixture was stirred for 2 min, then the reaction was quenched with methanol ($1cm^3$), followed by water ($10cm^3$). The solvents were evaporated. The residue was dissolved in de-ionised water ($700cm^3$) and purified by ion-exchange chromatography on Q Sepharose fast flow, eluting with a gradient of triethylammonium bicarbonate buffer (0–1M), pH 8.1 . The triethylammonium salt of (**276**) eluted between 610–660 mM buffer; $[\alpha]_D$ +34.2 (*c* 0.6 calc. for free acid, TEAB, pH 9.0); δ_H (D_2O, pH *ca.* 4; 400MHz) 3.52–3.89 (9 H, m, 2-H, 3-H or 4-H, 5-H, 6-H, 6-H', OCH_2CH_2O), 4.23 (1 H, q, *J* 9, 3-H or 4-H), 4.87 (1 H, d, *J* 3.7, 1-H); δ_P (D_2O, pH *ca.* 4; 162MHz) 0.94 (d, J_{HP} 8.3, ring phosphate), 1.43 (d, J_{HP} 9.8, ring phosphate), 45.50 (t, *J* 7.5,

$OCH_2CH_2OP[S]O_2$); *m/z*: (FAB$^-$) 479 [(M–1)$^-$, 100%]; (Found: M$^-$, 478.957. Calc. for $C_8H_{18}O_{15}SP_3$ [M-H]$^-$: 478.958).

6.5.19 Fluorescent Labelling of (276)

An aliquot of (**276**) equivalent to 8μmol by Briggs phosphate assay was freeze-dried then dissolved in degassed ethanol (0.05cm^3). A solution of *N*-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-*s*-indacene-3-propionyl)-*N'*-iodoacetyl ethylenediamine (**275**) (5mg, 10.0μmol, Cambridge BioScience) in degassed ethanol (0.45cm^3) was added and the resultant orange suspension was stirred at room temperature for 2 h, when ^{31}P NMR spectroscopy showed peaks at δ_P 16.5, 1.3 and 0.9ppm. The system was diluted with de-ionised water (50cm^3) and purified by ion-exchange chromatography on Q Sepharose fast flow, eluting with a gradient of triethylammonium bicarbonate buffer (0–1M), pH 7.5. The triethylammonium salt of *S*-[*N*-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-*s*-indacene-3-propionyl)-*N'*-acetyl ethylenediamine]-(2-hydroxyethyl) α-D-glucopyranoside 3,4-bisphosphate-2'-phosphorothioate (**277**) eluted at 580–650mM buffer; δ_P (D_2O, pH *ca.* 5, 162MHz) 0.55 (d, J_{HP} 8.8, ring phosphate), 3.72 (d, J_{HP} 10.3, ring phosphate), 18.71 (quintet, J_{HP} *ca.* 7, $OCH_2CH_2OP[O]_2SCH_2$); δ_F (D_2O, pH *ca.* 5, 376 MHz) –122.58 (s).

APPENDIX

NOMENCLATURE AND NUMBERING OF INOSITOLS, CARBOHYDRATES AND CYCLOHEXANONE FERRIER PRODUCTS

A.1 Inositols

The IUPAC Commission on the Nomenclature of Organic Chemistry (CNOC) has issued two documents concerned with nomenclature of cyclitols, the Tentative Cyclitol Nomenclature Rules,[312] and the updated 1973 Recommendations for Cyclitol.[313]

Under these recommendations, a cyclitol is defined as a cycloalkane containing one hydroxyl group on each of three or more ring atoms. The 1,2,3,4,5,6-cyclohexanehexols are termed generically "inositols," and are differentiated by an italicised prefix. There are nine possible inositols, illustrated with conventional numbering in fig. A1.

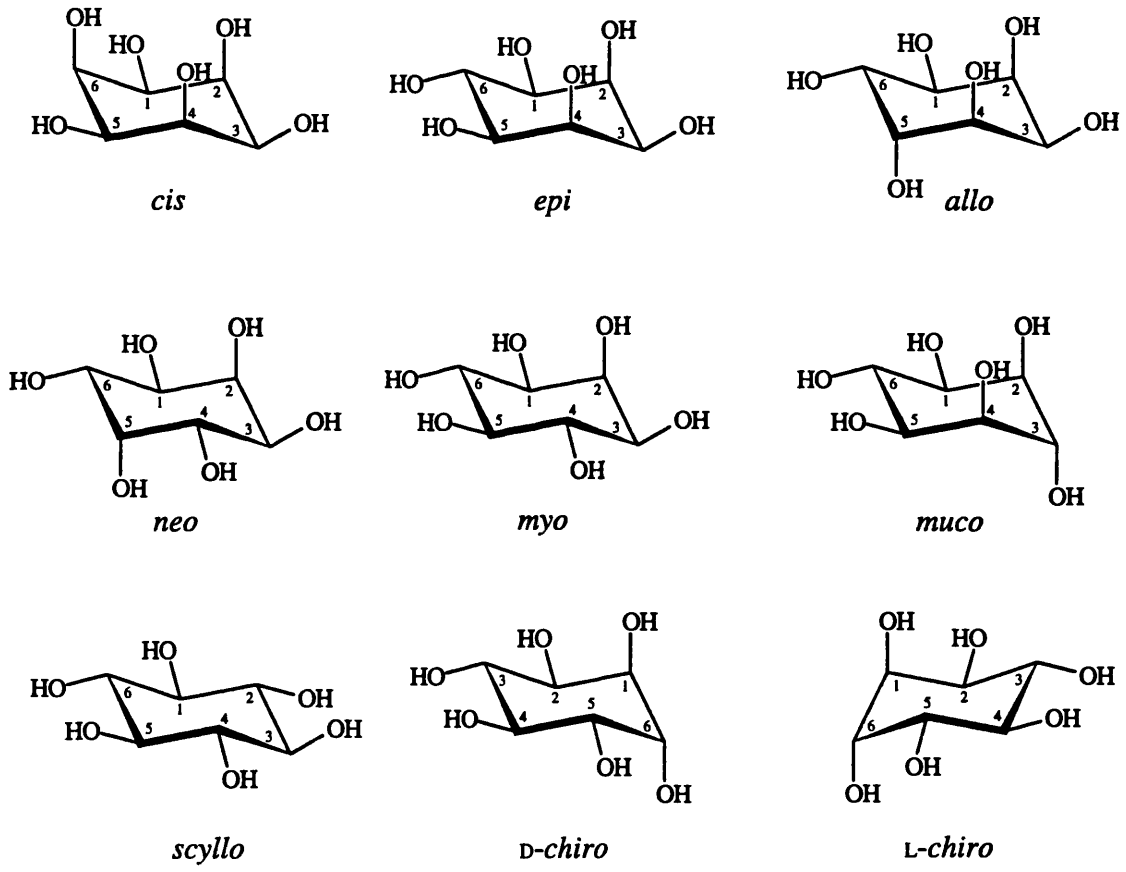
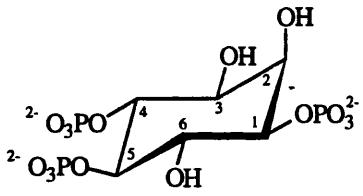


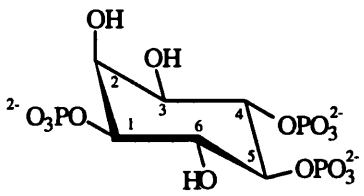
Fig. A.1 The Nine Inositols

Only D- and L-*chiro*-inositol are chiral, the other seven being *meso* compounds. However, on substitution the symmetry of these compounds may be disrupted. In this case usual (additive) rules for naming the derivative are followed,[314] and the compound is numbered such that substituents receive the lowest possible locants; anticlockwise numbering gives a substituted inositol with a D- prefix, clockwise with an L- prefix. This is exemplified by D- and L-*myo*-inositol 1,4,5-trisphosphate (**3a** and **3b**).



(3a)

D-*myo*-inositol-1,4,5-trisphosphate
(anticlockwise numbering)



(3b)

L-*myo*-inositol-1,4,5-trisphosphate
(clockwise numbering)

Often, particularly in metabolic sequences, systematic nomenclature is not applied, for reasons of clarity. For example, describing the dephosphorylation of D-*myo*-inositol 1,3,4-trisphosphate to L-*myo*-inositol 1,6-bisphosphate is somewhat more confusing than to D-*myo*-inositol 3,4-bisphosphate. This relaxation of systematic rules has been accepted by the Nomenclature Committee of the International Union of Biology (NC-IUB).[315]

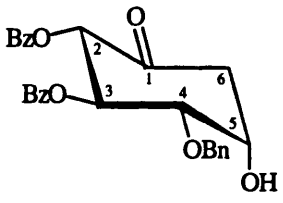
A widely adopted notation for referring to inositol polyphosphates and phosphorothioates, which has been accepted by the NC-IUB,[315] uses "Ins" to denote *myo*-inositol, followed by substituted positions in brackets and P_x (for x phosphate groups). Phosphorothioate groups are denoted by S. Absence of a configurational prefix implies the D-enantiomer. Thus, (**3a**) is represented as Ins(1,4,5)P_3, D-*myo*-inositol-1,4,5-trisphosphorothioate as Ins(1,4,5)PS_3, and D-*myo*-inositol-1,4-bisphosphate-5-phosphorothioate as Ins(1,4,5)P_3-5S. Such notation is used throughout this thesis.

A.2 Carbohydrates

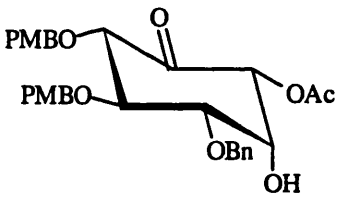
The nomenclature of carbohydrates is the subject of the CNOC document "Tentative Rules for Carbohydrate Nomenclature."[316] These comprehensive rules specify that substituted glycosides shall be named from the parent monosaccharide with the suffix "pyranoside" to denote a six-membered ring, and shall be numbered from the anomeric carbon.

A.3 Cyclohexanone Ferrier Products

Under the 1973 Recommendations for Cyclitol,[313] products of the Ferrier rearrangement such as (**148**) are named as derivatives of cyclohexanone. Numbering commences at the carbonyl carbon and proceeds in the direction to give substituents lowest possible locants. Thus, in (**148**) the deoxy position becomes position 6. Several methods are acceptable to specify the configuration of substituents in the ring. In this thesis, the Cahn-Ingold-Prelog *RS* nomenclature[314] is used for each position (the same convention is used in nomenclature of cyclopentane derivatives). Thus, (**148**) is named as (2*S*, 3*R*, 4*S*, 5*S*)-2,3-dibenzoyloxy-4-benzyloxy-5-hydroxycyclohexanone.

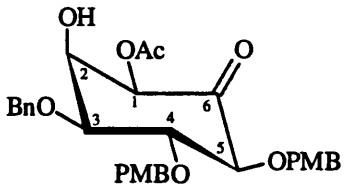


(**148**)



(**225**)

≡



Products of the modified Ferrier rearrangement, which give non-deoxy inososes such as (**225**) are named from the parent inositol and usual additive rules for substituents are followed. Compound (**225**) is therefore named as D-1-*O*-acetyl-3-*O*-benzyl-4,5-di-*O*-(*p*-methoxybenzyl)-*myo*-inos-6-ose.

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